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(54) **THERMOPHILIC AMINO ACID BIOSYNTHESIS SYSTEM ENZYME GENE OF THERMOTOLERANT CORYNEFORM BACTERIUM**

(57) A plurality of primer sets are designed based on a region where conservation at the amino acid level is observed among various microorganisms for known gene sequences corresponding to a gene coding for an enzyme of the L-amino acid biosynthetic pathway derived from *Corynebacterium thermoaminogenes*, preferably an enzyme that functions at a higher temperature compared with that of *Corynebacterium glutamicum*.

PCR is performed by using the primers and chromosomal DNA of *Corynebacterium thermoaminogenes* as a template. The primers with which an amplification fragment has been obtained are used as primers for screening to select a clone containing a target DNA fragment from a plasmid library of chromosomal DNA of *Corynebacterium thermoaminogenes*.

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Description

Technical Field

[0001] The present invention relates to heat resistant enzyme genes, in particular, genes for enzymes of biosynthetic pathway and uptake system of L-amino acids such as L-glutamic acid, of *Corynebacterium thermoaminogenes*, which is a thermophilic coryneform bacterium.

Background Art

[0002] The current main stream of the production of L-amino acids such as L-glutamic acid is the fermentative production utilizing coryneform bacteria. As for the fermentative production of L-amino acids, it has been attempted to reduce the cost based on breeding of strains with superior productivity and development of fermentation techniques. Although conventional attempts for realizing the cost reduction were mainly directed to achieving higher yield, energy required for cooling the fermentation heat generated during the culture cannot be ignored in addition to the raw material as the factors concerning the fermentation cost. That is, as for usual microorganisms used for the fermentation, the temperature of the medium rises due to fermentation heat generated by the microorganism themselves during the fermentation, and hence enzymes required for the fermentation may be inactivated or the productive bacteria may be killed. Therefore, it is necessary to cool the medium during the fermentation. Accordingly, in order to reduce the cooling cost, fermentation at high temperatures has been studied for many years. Moreover, if high temperature fermentation becomes possible, the reaction rate may also be improved. However, as for the L-amino acid fermentation, effective high temperature culture has not been realized so far.

[0003] *Corynebacterium thermoaminogenes* is a bacterium classified into coryneform bacteria like *Corynebacterium glutamicum* (*Brevibacterium lactofermentum*), which is commonly used for the fermentation of L-amino acids. However, it shows the optimum growth temperature of 37-43°C, which is higher than that of *Corynebacterium glutamicum*, i.e., 30-35°C, and shows the optimum temperature for L-glutamic acid production of 42-45°C, which is considerably shifted to the high temperature region (Japanese Patent Laid-open (Kokai) No. 63-240779/1988).

[0004] Meanwhile, there have been developed techniques for enhancing L-amino acid producing ability of *Corynebacterium* and *Brevibacterium* bacteria by introducing a gene coding for an L-amino acid synthesis system enzyme derived from *Escherichia coli* or *Corynebacterium glutamicum* into them. Examples of such an enzyme include, for example, citrate synthase (Japanese Patent Publication (Kokoku) No. 7-121228/1995), which is an enzyme of the L-glutamic acid biosynthetic pathway, glutamate dehydrogenase (Japanese Patent Laid-open No. 61-268185/1986), isocitrate dehydrogenase, aconitate hydratase (Japanese Patent Laid-open No. 63-214189) and so forth.

[0005] However, any L-amino acid biosynthesis enzymes and genes coding for them derived from thermophilic coryneform bacteria have not been reported.

Disclosure of the Invention

[0006] An object of the present invention is to provide genes coding for enzymes derived from *Corynebacterium thermoaminogenes*, preferably enzymes that function at a temperature higher than those of *Corynebacterium glutamicum*.

[0007] The inventors of the present invention extensively studied in order to achieve the aforementioned object. As a result, they successfully isolated genes coding for enzymes of the amino acid biosynthetic pathway of *Corynebacterium thermoaminogenes*, or genes coding for proteins involved in the uptake of amino acids into cells, and thus achieved the present invention.

[0008] That is, the present invention provides the followings.

- (1) A protein having the amino acid sequence of SEQ ID NO: 2 or the amino acid sequence of SEQ ID NO: 2 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has isocitrate lyase activity and shows 30% or more of residual activity after a heat treatment at 50°C for 5 minutes.
- (2) A protein having the amino acid sequence of SEQ ID NO: 4 or the amino acid sequence of SEQ ID NO: 4 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which is involved in acyl Co-A carboxylase activity derived from *Corynebacterium thermoaminogenes*.
- (3) A protein having the amino acid sequence of SEQ ID NO: 6 or the amino acid sequence of SEQ ID NO: 6 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has DtsR activity derived from *Corynebacterium thermoaminogenes*.
- (4) A protein having the amino acid sequence of SEQ ID NO: 8 or the amino acid sequence of SEQ ID NO: 8 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has

DtsR activity derived from *Corynebacterium thermoaminogenes*.

(5) A protein having the amino acid sequence of SEQ ID NO: 10 or the amino acid sequence of SEQ ID NO: 10 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which shows phosphofructokinase activity at 60°C in an equivalent or higher degree compared with the activity at 30°C.

(6) A protein having the amino acid sequence of SEQ ID NO: 94 or the amino acid sequence of SEQ ID NO: 94 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has activity for imparting sucrose assimilating ability to *Corynebacterium thermoaminogenes*.

(7) A protein having any one of the amino acid sequences of SEQ ID NOS: 17-20 or the amino acid sequence of any one of SEQ ID NOS: 17-20 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has a function involved in glutamic acid uptake and derived from *Corynebacterium thermoaminogenes*.

(8) A protein having the amino acid sequence of SEQ ID NO: 22 or the amino acid sequence of SEQ ID NO: 22 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has pyruvate dehydrogenase activity derived from *Corynebacterium thermoaminogenes*.

(9) A protein having the amino acid sequence of SEQ ID NO: 24 or the amino acid sequence of SEQ ID NO: 24 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has pyruvate carboxylase activity derived from *Corynebacterium thermoaminogenes*.

(10) A protein having the amino acid sequence of SEQ ID NO: 26 or the amino acid sequence of SEQ ID NO: 26 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has phosphoenolpyruvate carboxylase activity and shows 50% or more of residual activity after a heat treatment at 45°C for 5 minutes.

(11) A protein having the amino acid sequence of SEQ ID NO: 28 or the amino acid sequence of SEQ ID NO: 28 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has aconitase activity and shows 30% or more of residual activity after a heat treatment at 50°C for 3 minutes.

(12) A protein having the amino acid sequence of SEQ ID NO: 30 or the amino acid sequence of SEQ ID NO: 30 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has isocitrate dehydrogenase activity and shows 50% or more of residual activity after a heat treatment at 45°C for 10 minutes.

(13) A protein having the amino acid sequence of SEQ ID NO: 32 or the amino acid sequence of SEQ ID NO: 32 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has dihydrolipoamide dehydrogenase activity derived from *Corynebacterium thermoaminogenes*.

(14) A protein having the amino acid sequence of SEQ ID NO: 34 or the amino acid sequence of SEQ ID NO: 34 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has 2-oxoglutarate dehydrogenase activity and shows 30% or more of residual activity after a heat treatment at 50°C for 10 minutes.

(15) A protein having the amino acid sequence of SEQ ID NO: 80 in Sequence Listing or the amino acid sequence of SEQ ID NO: 80 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which shows glutamate dehydrogenase activity at 42°C in an equivalent or higher degree compared with the activity at 37°C.

(16) A protein having the amino acid sequence of SEQ ID NO: 90 in Sequence Listing or the amino acid sequence of SEQ ID NO: 90 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which shows citrate synthase activity at 37°C in an equivalent or higher degree compared with the activity at 23°C.

(17) A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 2 or the amino acid sequence of SEQ ID NO: 2 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having isocitrate lyase activity.

(18) The DNA according to (17), which is a DNA defined in the following (a1) or (b1):

(a1) a DNA which comprises the nucleotide sequence of SEQ ID NO: 1 in Sequence Listing.

(b1) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 1 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having isocitrate lyase activity.

(19) A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 4 or the amino acid sequence of SEQ ID NO: 4 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and involved in acyl Co-A carboxylase activity.

(20) The DNA according to (19), which is a DNA defined in the following (a2) or (b2):

- (a2) a DNA which comprises the nucleotide sequence of SEQ ID NO: 3 in Sequence Listing,
 (b2) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 3 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein involved in acyl Co-A carboxylase activity.

(21) A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 6 or the amino acid sequence of SEQ ID NO: 6 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having DtsR activity.

(22) The DNA according to (21), which is a DNA defined in the following (a3) or (b3):

- (a3) a DNA which comprises the nucleotide sequence of SEQ ID NO: 5 in Sequence Listing,
 (b3) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 5 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having DtsR activity.

(23) A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 8 or the amino acid sequence of SEQ ID NO: 8 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having DtsR activity.

(24) The DNA according to (23), which is a DNA defined in the following (a4) or (b4):

- (a4) a DNA which comprises the nucleotide sequence of SEQ ID NO: 7 in Sequence Listing,
 (b4) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 7 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having DtsR activity.

(25) A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 10 or the amino acid sequence of SEQ ID NO: 10 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having phosphofructokinase activity.

(26) The DNA according to (25), which is a DNA defined in the following (a5) or (b5):

- (a5) a DNA which comprises the nucleotide sequence of SEQ ID NO: 9 in Sequence Listing,
 (b5) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 9 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having phosphofructokinase activity.

(27) A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 93 or the amino acid sequence of SEQ ID NO: 93 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having invertase activity.

(28) The DNA according to (27), which is a DNA defined in the following (a6) or (b6):

- (a6) a DNA which comprises the nucleotide sequence of SEQ ID NO: 93 in Sequence Listing,
 (b6) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 93 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having invertase activity.

(29) A DNA which codes for a protein having any one of the amino acid sequences of SEQ ID NOS: 17-20 or the amino acid sequence of any one of SEQ ID NOS: 17-20 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having a function involved in glutamic acid uptake.

(30) The DNA according to (29), which is a DNA defined in the following (a7) or (b7):

- (a7) a DNA which comprises the nucleotide sequence of SEQ ID NO: 16 in Sequence Listing,
 (b7) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 16 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having a function involved in glutamic acid uptake.

(31) A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 22 or the amino acid sequence of SEQ ID NO: 22 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having pyruvate dehydrogenase activity.

(32) The DNA according to (31), which is a DNA defined in the following (a8) or (b8):

(a8) a DNA which comprises the nucleotide sequence of SEQ ID NO: 21 in Sequence Listing.

(b8) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 21 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having pyruvate dehydrogenase activity.

(33) A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 24 or the amino acid sequence of SEQ ID NO: 24 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having pyruvate carboxylase activity.

(34) A DNA according to (33), which is a DNA defined in the following (a9) or (b9):

(a9) a DNA which comprises the nucleotide sequence of SEQ ID NO: 23 in Sequence Listing.

(b9) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 23 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having pyruvate carboxylase activity.

(35) A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 26 or the amino acid sequence of SEQ ID NO: 26 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having phosphoenolpyruvate carboxylase activity.

(36) The DNA according to (35), which is a DNA defined in the following (a10) or (b10):

(a10) a DNA which comprises the nucleotide sequence of SEQ ID NO: 25 in Sequence Listing.

(b10) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 25 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having phosphoenolpyruvate carboxylase activity.

(37) A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 28 or the amino acid sequence of SEQ ID NO: 28 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having aconitase activity.

(38) The DNA according to (37), which is a DNA defined in the following (a11) or (b11):

(a11) a DNA which comprises the nucleotide sequence of SEQ ID NO: 27 in Sequence Listing.

(b11) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 27 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having aconitase activity.

(39) A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 30 or the amino acid sequence of SEQ ID NO: 30 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having isocitrate dehydrogenase activity.

(40) The DNA according to (39), which is a DNA defined in the following (a12) or (b12):

(a12) a DNA which comprises the nucleotide sequence of SEQ ID NO: 27 in Sequence Listing.

(b12) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 27 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having isocitrate dehydrogenase activity.

(41) A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 32 or the amino acid sequence of SEQ ID NO: 32 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having dihydrolipoamide dehydrogenase activity.

(42) The DNA according to (41), which is a DNA defined in the following (a13) or (b13):

(a13) a DNA which comprises the nucleotide sequence of SEQ ID NO: 31 in Sequence Listing.

(b13) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 31 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having dihydrolipoamide dehydrogenase activity.

(43) A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 34 or the amino acid

sequence of SEQ ID NO: 34 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having 2-oxoglutarate dehydrogenase activity.

(44) The DNA according to (43), which is a DNA defined in the following (a14) or (b14):

(a14) a DNA which comprises the nucleotide sequence of SEQ ID NO: 33 in Sequence Listing.

(b14) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 33 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having 2-oxoglutarate dehydrogenase activity.

(45) A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 80 in Sequence Listing or the amino acid sequence of SEQ ID NO: 80 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and showing glutamate dehydrogenase activity at 42°C in an equivalent or higher degree compared with the activity at 37°C.

(46) The DNA according to (45), which is a DNA defined in the following (a15) or (b15):

(a15) a DNA which comprises the nucleotide sequence of SEQ ID NO: 79 in Sequence Listing.

(b15) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 79 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein showing glutamate dehydrogenase activity at 42°C in an equivalent or higher degree compared with the activity at 37°C.

(47) A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 90 in Sequence Listing or the amino acid sequence of SEQ ID NO: 90 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and showing citrate synthase activity at 37°C in an equivalent or higher degree compared with the activity at 23°C.

(48) The DNA according to (47), which is a DNA defined in the following (a16) or (b16):

(a16) a DNA which comprises the nucleotide sequence of SEQ ID NO: 89 in Sequence Listing.

(b16) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 89 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein showing citrate synthase activity at 37°C in an equivalent or higher degree compared with the activity at 23°C.

(49) A method for producing L-amino acid, which comprises culturing a microorganism introduced with a DNA according to any one of (17) to (48) in a medium to produce and accumulate L-amino acid in the medium, and collecting the L-amino acid from the medium.

[0009] The term "DNA of the present invention" is used hereinafter for referring to either one or all of the aforementioned DNAs.

[0010] Hereafter, the present invention will be explained in detail.

[0011] The nucleotide sequences of the DNA of the present invention, names of the genes, and the proteins encoded by the DNA of the present invention are shown in Table 1.

Table 1

Nucleotide sequence	Name of gene	Encoded protein (abbreviation)
SEQ ID NO: 1	<i>aceA</i>	Isocitrate lyase (ICL)
SEQ ID NO: 3	<i>accBC</i>	acyl Co-A carboxylase BC subunit
SEQ ID NO: 5	<i>dtsR1</i>	DTSR1 protein
SEQ ID NO: 7	<i>dtsR2</i>	DTSR2 protein
SEQ ID NO: 9	<i>pfk</i>	Phosphofructokinase
SEQ ID NOS: 11, 13, 15, 93	<i>scrB</i>	Invertase
SEQ ID NO: 16	<i>gluABCD</i>	glutamic acid uptake system
SEQ ID NO: 21	<i>pdhA</i>	pyruvate dehydrogenase
SEQ ID NO: 23	<i>pc</i>	pyruvate carboxylase
SEQ ID NO: 25	<i>ppc</i>	phosphoenolpyruvate carboxylase
SEQ ID NO: 27	<i>acn</i>	aconitase

Table 1 (continued)

Nucleotide sequence	Name of gene	Encoded protein (abbreviation)
SEQ ID NO: 29	<i>icd</i>	isocitrate dehydrogenase
SEQ ID NO: 31	<i>lpd</i>	dihydrolipoamide dehydrogenase
SEQ ID NO: 33	<i>odhA</i>	2-oxoglutarate dehydrogenase
SEQ ID NO: 79	<i>gdh</i>	glutamate dehydrogenase
SEQ ID NO: 89	<i>gltA</i>	citrate synthase

[0012] The open reading frames (ORFs) of SEQ ID NOS: 3, 23, 25, 31 and 33 and the fourth ORF of SEQ ID NO: 16 all start from GTG. Although the amino acids encoded by these GTG are indicated as valine in Sequence Listing, they may be methionine.

[0013] The sequence of SEQ ID NO: 16 contains four ORFs, which correspond to *gluA*, *gluB*, *gluC* and *gluD* in this order from the 5' end side.

[0014] The aforementioned DNA sequences were isolated from chromosomal DNA of the *Corynebacterium thermoaminogenes* AJ12310 strain (FERM BP-1542). However, the DNA sequences shown in SEQ ID NOS: 11 and 13 were isolated from *Corynebacterium thermoaminogenes* AJ12340 strain (FERM BP-1539) and AJ12309 strain (FERM BP-1541), respectively, which had invertase activity and sucrose assimilating property, because the AJ12310 strain did not have invertase activity and sucrose assimilating property, and the *scrB* gene isolated from the strain had not any open reading frame.

[0015] The *Corynebacterium thermoaminogenes* AJ12310 strain (also referred to as YS-314 strain) and AJ12309 strain (also referred to as YS-155 strain) were deposited at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Ministry of International Trade and Industry (postal code: 305-8566, 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan) on March 13, 1987 and given deposition numbers of FERM P-9246 and FERM P-9245, respectively. Then, they were transferred to international depositions under the provisions of the Budapest Treaty on October 27, 1987, and given deposition numbers of FERM BP-1542 and FERM BP-1541, respectively.

[0016] The AJ12340 strain (also referred to as YS-40 strain) was deposited at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Ministry of International Trade and Industry (postal code: 305-8566, 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan) on March 10, 1987 and given a deposition number of FERM P-9277. Then, it was transferred to an international deposition under the provisions of the Budapest Treaty on October 27, 1987, and given a deposition number of FERM BP-1539.

[0017] The nucleotide sequences shown in SEQ ID NOS: 11, 13 and 15 are partial sequences of *scrB*, and the sequences of SEQ ID NOS: 11 and 13 code for partial amino acid sequences of invertase shown in SEQ ID NOS: 12 and 14.

[0018] A DNA sequence containing a partial fragment of a target gene can be obtained by comparing already reported nucleotide sequences for the target gene of various microorganisms such as *Brevibacterium lactofermentum* to select a region containing a well-conserved nucleotide sequence, and carrying out PCR using primers designed based on the nucleotide sequence of the region and chromosomal DNA of *Corynebacterium thermoaminogenes* as a template. Further, by performing hybridization using the obtained DNA fragment or a probe prepared based on the sequence of the fragment to screen a chromosomal DNA library of *Corynebacterium thermoaminogenes*, a DNA fragment containing the gene in its full length can be obtained. A DNA fragment containing the gene in its full length can also be obtained by performing genome walking using the obtained partial fragment of the gene. The genome walking can be carried out by using a commercially available kit, for example, TaKaRa LA PCR in vitro Cloning Kit (produced by Takara Shuzo).

[0019] For example, a partial sequence of DNA coding for glutamate dehydrogenase (henceforth the DNA is also referred to as "*gdh*", and the enzyme is also referred to as "GDH") can be obtained from chromosomal DNA of *Corynebacterium thermoaminogenes* such as the *Corynebacterium thermoaminogenes* AJ12310 strain by PCR (polymerase chain reaction) using the chromosomal DNA as a template and primers having the nucleotide sequences shown in SEQ ID NOS: 77 and 78 of Sequence Listing. Further, by performing genome walking using the obtained partial fragment, the whole *gdh* gene can be obtained.

[0020] Further, a partial sequence of DNA coding for citrate synthase (henceforth the DNA is also referred to as "*gltA*", and the enzyme is also referred to as "CS") can be obtained from chromosomal DNA of *Corynebacterium thermoaminogenes* such as the *Corynebacterium thermoaminogenes* AJ12310 strain by PCR (polymerase chain reaction) using the chromosomal DNA as a template and primers having the nucleotide sequences shown in SEQ ID NOS: 83 and 84 of Sequence Listing. Further, by performing genome walking using the obtained partial fragment, the whole *gltA* gene can be obtained.

[0021] The nucleotide sequences of the aforementioned primers were designed based on a nucleotide sequence in

a region containing a well-conserved nucleotide sequence among the already reported *gdh* genes or *gluA* genes of various microorganisms, which region was found by comparison of the genes.

[0022] As for DNA sequences coding for the other enzymes, partial fragments coding for those enzymes can be similarly obtained by using the primers mentioned in Table 1, and the genes in full length can be obtained by using the

[0023] While the DNA of the present invention was obtained as described above, it can also be obtained from a chromosomal DNA library of *Corynebacterium thermoaminogenes* by hybridization using an oligonucleotide prepared based on the nucleotide sequences of the DNA of the present invention as a probe.

[0024] Methods for preparation of chromosomal DNA, construction of chromosomal DNA library, hybridization, PCR, preparation of plasmid DNA, digestion and ligation of DNA, transformation and so forth are described in Sambrook, J., Fritsch, E.F., Maniatis, T., Molecular Cloning, Cold Spring Harbor Laboratory Press, 1.21 (1989). Further, genome walking can be performed by using a commercially available kit, for example, TaKaRa LA PCR in vitro Cloning Kit (produced by Takara Shuzo).

[0025] Specific methods for obtaining the DNA of the present invention will be explained hereafter.

[0026] First, chromosomal DNA of *Corynebacterium thermoaminogenes* is digested with a suitable restriction enzyme, for example, *Sau3AI*, and fractionated by agarose gel electrophoresis to obtain a DNA fragment of about 4 to 6 kb. The obtained DNA fragment is inserted into a cloning vector such as pHS399, and *Escherichia coli* is transformed with the obtained recombinant plasmid to produce a plasmid library of the chromosomal DNA.

[0027] Separately, primers are produced for use in selecting a clone containing a target gene from a plasmid library by PCR. These primers are designed based on conserved amino acid regions from various microorganisms corresponding to the gene of interest. In the design of primers, a plurality of primer sets are designed considering the codon usage of coryneform bacteria.

[0028] Then, in order to investigate propriety of the produced primers, PCR is performed by using these primers and chromosomal DNA of *Corynebacterium thermoaminogenes* as a template. Further, PCR is performed by using primers from which an amplification fragment has been obtained as primers for screening and a recombinant plasmid prepared from the plasmid library as a template to select a clone containing the target DNA fragment. This operation can be quickly carried out by performing the PCR for every batch including several tens of transformant strains as primary screening and performing colony PCR for the batch with which an amplification fragment was obtained as secondary screening. The fragment lengths of the amplified genes are shown in Tables 2 to 7.

[0029] If a transformant selected as described above contains a target gene is confirmed by preparing a recombinant DNA from the transformant selected as described above, determining the nucleotide sequence of the inserted fragment by the dideoxy termination method, and comparing the nucleotide sequence with a known gene sequence.

[0030] When the obtained DNA fragment contains a part of the target gene, the deleted part is obtained by genome walking.

[0031] The DNA of the present invention may code for a protein including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, so long as the encoded protein has its original function. The number meant by the term "several" may vary depending on positions in the three-dimensional structure of protein or kinds of amino acid residues. However, in general, such a protein preferably shows homology of 30 to 40% or more, more preferably 55 to 65% or more, with respect to a corresponding whole amino acid sequence of the protein. More specifically, the term "several" means a number of 2 to several hundreds, preferably 2 to several tens, more preferably 2 to 10.

[0032] Nucleotide and amino acid sequence were analyzed by, for example, the method developed by Lipman and Pearson (Science, 227, 1435-1441, 1985) by using commercially available software such as Genetyx-Mac computer program (Software Development Co., Tokyo, Japan).

[0033] GDH may be one showing homology of 40 to 80% or more, preferably 80 to 90% or more, for the total amino acid sequence constituting GDH, and showing GDH activity at 42°C equivalent to or higher than the activity at 37°C. In this case, the term "several" means a number of 2 to 30, preferably 2 to 50, more preferably 2 to 10.

[0034] CS may be one showing homology of 40 to 80% or more, preferably 80 to 90% or more, for the total amino acid sequence constituting CS, and showing CS activity at 37°C equivalent to or higher than the activity at 23°C. In this case, the term "several" means a number of 2 to 300, preferably 2 to 50, more preferably 2 to 10.

[0035] A DNA, which codes for the substantially same protein as the original protein as described above, can be obtained by, for example, modifying the nucleotide sequence, for example, by means of the site-directed mutagenesis so that one or more amino acid residues at a specific site should involve substitution, deletion, insertion, addition or inversion. A DNA modified as described above may also be obtained by a conventionally known mutation treatment. The mutation treatment includes a method for treating DNA coding for a target gene in vitro, for example, with hydroxylamine, and a method for treating a microorganism, for example, a bacterium belonging to the genus *Escherichia*, harboring DNA coding for the target gene with ultraviolet irradiation or a mutating agent usually used for the mutation treatment such as N-methyl-N'-nitro-N-nitrosoguanidine (NTG) and nitrous acid.

[0036] The substitution, deletion, insertion, addition, or inversion of nucleotides as described above also includes mutant or variant that naturally occurs due to the difference of strains of *Corynebacterium thermoaminogenes* or the like.

[0037] A DNA coding for substantially the same protein as the original protein can be obtained by expressing DNA having a mutation in an appropriate cell, and investigating activity or function of the expressed product protein. The DNA coding for substantially the same protein as the original protein can also be obtained by, for example, isolating a DNA which is hybridizable with a DNA having each of the nucleotide sequences of the sequences of which sequence numbers are mentioned in Table 1 or a coding region thereof, or a probe designed based on the nucleotide sequence under a stringent condition, and which codes for a protein having the activity originally possessed by the protein, from DNA coding for a protein having a mutation or from a cell harboring it. The activity preferably means each enzymatic activity at 42°C for GDH or 37°C for CS.

[0038] The aforementioned probe can be prepared from a DNA having any one of the nucleotide sequences of which sequence numbers are shown in Table 1 or a DNA having any one of the nucleotide sequences by PCR using suitable primers.

[0039] The "stringent condition" referred to herein is a condition under which so-called specific hybrid is formed, and non-specific hybrid is not formed. It is difficult to clearly express this condition by using any numerical value. However, for example, the stringent condition includes a condition under which DNAs having high homology, for example, DNAs having homology of not less than 50% are hybridized with each other, and DNAs having homology lower than the above are not hybridized with each other. Alternatively, the stringent condition is exemplified by a condition under which DNAs are hybridized with each other at a salt concentration corresponding to an ordinary condition of washing in Southern hybridization, i.e., 60°C, 1 x SSC, 0.1% SDS, preferably 0.1 x SSC, 0.1% SDS.

[0040] The gene, which is hybridizable under the condition as described above, includes those having a stop codon generated in the gene, and those having no activity due to mutation of active site. However, such genes can be easily removed by ligating the genes with a commercially available activity expression vector, and measuring the activity or function.

[0041] A protein corresponding to each DNA of the present invention can be produced by expressing the DNA in a suitable host-vector system.

[0042] As the host used for the expression of a gene, there can be mentioned various prokaryotic cells including *Brevibacterium lactofermentum* (*Corynebacterium glutamicum*), coryneform bacteria such as *Corynebacterium thermoaminogenes*, *Escherichia coli*, *Bacillus subtilis* and so forth, and various eucaryotic cells including *Saccharomyces cerevisiae*, animal cells and plant cells. Among these, prokaryotic cells, in particular, coryneform bacteria and *Escherichia coli* are preferred.

[0043] If the DNA of the present invention is ligated to a vector DNA autonomously replicable in cells of *Escherichia coli* and/or coryneform bacteria and so forth to form a recombinant DNA, and this recombinant DNA is introduced into an *Escherichia coli* cell, the subsequent procedure becomes easy. The vector autonomously replicable in *Escherichia coli* cells is preferably a plasmid vector autonomously replicable in the host cell, and examples thereof include pUC19, pUC18, pBR322, pHSG299, pHSG399, pHSG398, RSF1010 and so forth.

[0044] As the vector autonomously replicable in coryneform bacterium cells, there can be mentioned pAM330 (refer to Japanese Patent Laid-open No. 58-67699/1983), pHM1519 (refer to Japanese Patent Laid-open No. 58-77895/1983) and so forth. Moreover, if a DNA fragment having an ability to make a plasmid autonomously replicable in coryneform bacteria is taken out from these vectors and inserted into the aforementioned vectors for *Escherichia coli*, they can be used as a so-called shuttle vector autonomously replicable in both of *Escherichia coli* and coryneform bacteria.

[0045] Examples of such a shuttle vector include those mentioned below. There are also indicated microorganisms that harbor each vector, and accession numbers thereof at international depositories are shown in the parentheses, respectively.

pAJ655	<i>Escherichia coli</i> AJ11882 (FERM BP-136)
	<i>Corynebacterium glutamicum</i> SR8201 (ATCC39135)
pAJ1844	<i>Escherichia coli</i> AJ11883 (FERM BP-137)
	<i>Corynebacterium glutamicum</i> SR8202 (ATCC39136)
pAJ611	<i>Escherichia coli</i> AJ11884 (FERM BP-138)
pAJ3148	<i>Corynebacterium glutamicum</i> SR8203 (ATCC39137)
pAJ440	<i>Bacillus subtilis</i> AJ11901 (FERM BP-140)
pHC4	<i>Escherichia coli</i> AJ12617 (FERM BP-3532)

[0046] In order to prepare a recombinant DNA by ligating the DNA of the present invention and a vector that functions in coryneform bacteria, the vector is digested with a restriction enzyme that provides an end corresponding to an end of the DNA of the present invention. The ligation is normally attained by using a ligase such as T4 DNA ligase.

[0047] To introduce the recombinant DNA prepared as described above into a host such as coryneform bacteria,

any known transformation methods that have hitherto been reported can be employed. For instance, employable are a method of treating recipient cells with calcium chloride so as to increase the permeability for DNA, which has been reported for *Escherichia coli* K-12 (Mandel, M. and Higa, A., *J. Mol. Biol.*, 53, 159 (1970)), and a method of preparing competent cells from cells which are at the growth phase followed by introducing the DNA thereto, which has been reported for *Bacillus subtilis* (Duncan, C.H., Wilson, G.A. and Young, F.E., *Gene*, 1, 153 (1977)). In addition to these, also employable is a method of making DNA-recipient cells into protoplasts or spheroplasts, which can easily take up recombinant DNA, followed by introducing the recombinant DNA into the cells, which is known to be applicable to *Bacillus subtilis*, actinomycetes and yeasts (Chang, S. and Choen, S.N., *Molec. Gen. Genet.*, 168, 111 (1979); Bibb, M.J., Ward, J.M. and Hopwood, O.A., *Nature*, 274, 398 (1978); Hinnen, A., Hicks, J.B. and Fink, G.R., *Proc. Natl. Sci. USA*, 75, 1929 (1978)). The transformation of coryneform bacteria can be effectively performed by the electric pulse method (refer to Japanese Patent Laid-open No. 2-207791).

[0048] As for the transformation of thermophilic coryneform bacteria such as *Corynebacterium thermoaminogenes*, it can be efficiently performed by treating cells with an agent that changes the structure of cell walls of the host cells, and applying an electric pulse to a solution containing DNA and the cells of which structure of the cell walls have been changed. The aforementioned agent is an agent that can change the structure of cell walls so that the cells can uptake the DNA when an electric pulse is applied to a solution containing the cells treated with the agent and the DNA (henceforth also referred to as a "cell wall treatment agent"). Examples of such an agent include agents that inhibit normal synthesis of bacterial cell wall and agents that lyse bacterial cell walls. Specific examples thereof include lysozyme, penicillin G, glycine and so forth.

[0049] Those cell wall treatment agents may be used each alone, or two or more kinds of them may be used in combination. Among the aforementioned agents, lysozyme and penicillin G are preferred, and lysozyme is particularly preferred.

[0050] Furthermore, the transformation of *Corynebacterium thermoaminogenes* can also be performed by applying an electric pulse to a solution containing DNA and the host cells of which cell walls has been weakened by a physical method such as ultrasonication (*FEMS Microbiology Letters*, 151, 135-138 (1987)).

[0051] In order to efficiently express a gene contained in the DNA of the present invention, a promoter that functions in the host cell such as lac, trp and P_L may be ligated upstream from the coding region of the gene. If a vector containing a promoter is used as the vector, ligation of each gene, vector and promoter can be attained by one step.

[0052] The proteins of the present invention, which can be produced as described above, can be purified as required from a cell extract or medium by using usual methods for purifying enzymes such as ion exchange chromatography, gel filtration chromatography, adsorption chromatography, salting out and solvent precipitation.

[0053] It is expected that the proteins of the present invention are excellent in thermal stability or exhibit higher activity at high temperatures compared with the corresponding proteins of *Corynebacterium glutamicum* and so forth. For example, GDH of *Brevibacterium lactofermentum* shows the highest GDH specific activity around 37°C, and the activity is markedly reduced around 42°C. However, GDH of the present invention shows at 42°C the GDH activity equivalent to or higher than the activity at 37°C. In a preferred embodiment, GDH of the present invention shows the highest specific activity around 42°C, and shows the activity even at 45°C.

[0054] The GDH activity can be measured by, for example, adding the enzyme to 100 mM Tris-HCl (pH 8.0), 20 mM NH_4Cl , 10 mM sodium α -ketoglutarate, 0.25 mM NADPH, and determining change of absorbance at 340 nm (*Molecular Microbiology* 6, 317-326 (1992)).

[0055] Further, CS of *Brevibacterium lactofermentum* shows the highest CS specific activity around 23°C, and the activity is markedly reduced around 33°C. To the contrary, CS of the present invention shows at 37°C the CS activity equivalent to or higher than the activity at 23°C. In a preferred embodiment, CS of the present invention shows reaction temperature-dependently higher activity up to around 37°C, and shows, even at 40°C, about 40% of the activity with respect to the activity at 37°C.

[0056] The CS activity can be measured by, for example, the method described in *Methods in Enzymol.*, 13, 3-11 (1969).

[0057] Further, other proteins of the present invention typically have the following characteristics. The isocitrate lyase has 30% or more of residual activity after a heat treatment at 50°C for 5 minutes. The phosphofructokinase has, at 60°C, the activity equivalent to or higher than the activity at 30°C. The phosphoenolpyruvate carboxylase has 50% or more of residual activity after a heat treatment at 45°C for 5 minutes. The aconitase has 30% or more of residual activity after a heat treatment at 50°C for 3 minutes. The isocitrate dehydrogenase has 50% or more of residual activity after a heat treatment at 45°C for 10 minutes. The 2-oxoglutarate dehydrogenase has 30% or more of residual activity after a heat treatment at 50°C for 10 minutes.

[0058] The proteins of the present invention can also be obtained from cell extracts of *Corynebacterium thermoaminogenes* such as the *Corynebacterium thermoaminogenes* AJ12310 strain by using each activity as an index and usual purification methods for purifying enzymes such as ion exchange chromatography, gel filtration chromatography, adsorption chromatography, salting out and solvent precipitation.

[0059] Among the DNA of the present invention, *pfk*, *pdhA*, *pc*, *ppc*, *acn*, *icd*, *gdh* and *gluA* (names of the enzymes encoded by these are shown in Table 1) can be introduced into L-amino acid production bacteria such as coryneform bacteria to enhance their L-amino acid producing ability. It is also expected that coryneform bacteria introduced with the DNA of the present invention become possible to produce L-amino acid at a temperature higher than usual. The L-amino acid includes L-glutamic acid, L-aspartic acid, L-lysine, L-arginine, L-proline, L-glutamine and so forth.

[0060] For example, it is expected that L-glutamic acid production bacteria such as coryneform bacteria introduced with the *gdh* gene or *gluA* gene come to be able to produce L-glutamic acid at a temperature higher than usual. Further, although CS of *Brevibacterium lactofermentum* may not fully function at a usual culture temperature, for example, 31.5°C, the activity can be enhanced by introducing the *gluA* gene of the present invention.

[0061] Further, *dtsR1* and *dtsR2* are genes that code for proteins imparting resistance to surfactant to coryneform bacteria (DTSR protein), and coryneform L-glutamic acid producing bacteria of which these genes are disrupted produce a marked amount of L-glutamic acid even under a condition where biotin is present in such an amount that a wild strain becomes to be substantially unable to produce L-glutamic acid. Further, if *dtsR1* and *dtsR2* genes of coryneform L-glutamic acid producing bacteria having L-lysine producing ability are amplified, the bacteria are imparted with an ability to produce a marked amount of L-lysine (WO95/23224, Japanese Patent Laid-open (Kokai) No. 10-234371/1998).

[0062] The *scrB* gene can be used for improvement of coryneform bacteria for use in the production of L-amino acids by using coryneform bacteria in a medium containing sucrose.

[0063] By deleting *aceA*, *accBC*, *lpd* or *odhA* of L-glutamic acid producing coryneform bacteria and so forth, their L-glutamic acid productivity can be enhanced. Further, *gluABCD* is a gene cluster of the L-glutamic acid uptake system, and by deleting one to four of *gluA*, *gluB*, *gluC* and *gluD* in coryneform L-glutamic acid producing bacteria, the amount of L-glutamic acid accumulated in the medium can be increased. *aceA*, *accBC*, *lpd*, *odhA* and *gluABCD* of the present invention can be used for disruption of these genes on chromosome.

[0064] The medium used for producing L-amino acids by utilizing a microorganism introduced with the DNA of the present invention may be a usual medium that contains a carbon source, a nitrogen source, inorganic ions and other organic trace nutrients as required. As the carbon source, there can be used hydrocarbons such as glucose, lactose, galactose, fructose, sucrose, blackstrap molasses and starch hydrolysate; alcohols such as ethanol and inositol; or organic acids such as acetic acid, fumaric acid, citric acid and succinic acid.

[0065] As the nitrogen source, there can be used inorganic ammonium salts such as ammonium sulfate, ammonium nitrate, ammonium chloride, ammonium phosphate and ammonium acetate, ammonia, organic nitrogen such as peptone, meat extract, yeast extract, corn steep liquor and soybean hydrolysate, ammonia gas, aqueous ammonia and so forth.

[0066] As the inorganic ions (or sources thereof), added is a small amount of potassium phosphate, magnesium sulfate, iron ions, manganese ions and so forth. As for the organic trace nutrients, it is desirable to add required substances such as vitamin B₁, yeast extract and so forth in a suitable amount as required.

[0067] The culture is preferably performed under an aerobic condition attained by shaking, stirring for aeration or the like for 16 to 72 hours. The culture temperature is controlled to be at 30°C to 47°C, and pH is controlled to be 5 to 9 during the culture. As for the culture temperature, the culture may be performed at a temperature suitable for culture of a microorganism not introduced with the DNA of the present invention or a temperature higher than that. For adjustment of pH, inorganic or organic acidic or alkaline substances, ammonia gas and so forth can be used.

[0068] Collection of L-amino acids from fermentation broth can be attained by a combination of known methods such as techniques utilizing ion exchange resin, precipitation, crystallization and so forth depending on the kind of the L-amino acids.

Brief Explanation of the Drawings

[0069] Fig. 1 shows variation with temperature in activity of glutamate dehydrogenases derived from the *Corynebacterium thermoaminogenes* AJ12310 strain and the *Brevibacterium lactofermentum* 2256 strain.

[0070] Fig. 2 shows thermal stability of glutamate dehydrogenases derived from the AJ12310 strain and the 2256 strain.

[0071] Fig. 3 shows variation with temperature in activity of citrate synthases derived from the AJ12310 strain and the 2256 strain.

[0072] Fig. 4 shows thermal stability of citrate synthases derived from the AJ12310 strain and the 2256 strain.

[0073] Fig. 5 shows variation with temperature in activity of isocitrate lyases derived from the AJ12310 strain and the 2256 strain.

[0074] Fig. 6 shows thermal stability of isocitrate lyases derived from the AJ12310 strain and the 2256 strain.

[0075] Fig. 7 shows variation with temperature in activity of phosphofructokinases derived from the AJ12310 strain and the 2256 strain.

[0076] Fig. 8 shows thermal stability of phosphofructokinases derived from the AJ12310 strain and the 2256 strain.

[0077] Fig. 9 shows variation with temperature in activity of phosphoenolpyruvate carboxylases derived from the AJ12310 strain and the 2256 strain.

[0078] Fig. 10 shows thermal stability of phosphoenolpyruvate carboxylases derived from the AJ12310 strain and the 2256 strain.

[0079] Fig. 11 shows variation with temperature in activity of aconitases derived from the AJ12310 strain and the 2256 strain.

[0080] Fig. 12 shows thermal stability of aconitases derived from the AJ12310 strain and the 2256 strain.

[0081] Fig. 13 shows variation with temperature in activity of isocitrate dehydrogenases derived from the AJ12310 strain and the 2256 strain.

[0082] Fig. 14 shows thermal stability of isocitrate dehydrogenases derived from the AJ12310 strain and the 2256 strain.

[0083] Fig. 15 shows thermal stability of 2-oxoglutarate dehydrogenases derived from the AJ12310 strain and the 2256 strain.

[0084] Fig. 16 shows construction of plasmid pSCR155 carrying *scrB* gene.

[0085] Fig. 17 shows construction of plasmid pPDHA-2 carrying *pdhA* gene.

[0086] Fig. 18 shows L-glutamic acid productivity of a *pdhA* gene-amplified strain: (a) 37°C and (b) 44°C.

[0087] Fig. 19 shows construction of a plasmid pICD-4 carrying *icd* gene.

[0088] Fig. 20 shows L-glutamic acid productivity of an *icd* gene-amplified strain: (a) 37°C and (b) 44°C.

[0089] Fig. 21 shows construction of plasmids pHSG299YGDH and pYGDH.

[0090] Fig. 22 shows construction of plasmids pHSG299YCS and pYCS.

Best Mode for Carrying out the Invention

[0091] Hereafter, the present invention will be further specifically explained with reference to the following examples.

Example 1

<1> Production of plasmid library of *Corynebacterium thermoaminogenes*

[0092] The *Corynebacterium thermoaminogenes* AJ12310 strain was cultured in CM2B liquid medium (1 g/dl of yeast extract (produced by Difco), 1 g/dl of polypeptone (produced by Nippon Seiyaku), 0.5 g/dl of NaCl, 10 µg/dl of biotin, pH 7.0 (adjusted with KOH)) at 37°C for 15 hours, and its chromosomal DNA was prepared from the 10 ml of the medium by using a chromosomal DNA extraction kit (Bacterial Genome DNA Purification Kit (produced by Advanced Genetic Technologies)). The obtained DNA was partially digested with a restriction enzyme *Sau3A*I, and subjected to 0.8% agarose gel electrophoresis to fractionate the DNA. Then, a band corresponding to a DNA fragment of about 4 to 6 kb was excised from the gel, and a DNA fragment of the objective size was obtained by using a DNA gel extraction kit (GIBCO BRL, Concert™ Rapid Gel Extraction System).

[0093] The plasmid pHSG399 (produced by Takara Shuzo) was fully digested with *Bam*HI, and its end was dephosphorylated by using alkaline phosphatase (CIAP; produced by Takara Shuzo). This vector fragment and the aforementioned chromosomal DNA fragment were ligated by using a DNA ligation kit produced by Takara Shuzo, and *Escherichia coli* JM109 was transformed with the obtained recombinant vector. Selection of transformants was performed on LB agar medium (containing 1.5 g/dl of agar) containing 30 µg/ml of chloramphenicol, 0.04 mg/ml of IPTG (isopropyl-β-D-thiogalactopyranoside) and 0.04 mg/ml of X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) to obtain about 4000 white colonies.

<2> Design of primers for amplification of each gene

[0094] Primers for use in selection of a clone containing each target gene by PCR from the plasmid library obtained above were designed. The target genes were mentioned above.

[0095] The primers were designed based on a known gene sequence of coryneform bacteria, i.e., its sequence of a region where conservation at the amino acid level was observed when compared with corresponding genes of other microorganisms. Considering the codon usage of coryneform bacteria, a plurality of primer sets were designed for each gene.

[0096] To examine propriety of the prepared primers, PCR was performed by using these primers and chromosomal DNA of the *Corynebacterium thermoaminogenes* AJ12310 strain as a template to amplify each gene fragment. As a result, when the PCR was performed by using the primers shown in the upper rows of Tables 2 to 7 under the conditions indicated as "PCR conditions for obtaining partial fragment" in the tables, an amplified fragment was observed for all

of the genes. The parenthesized numbers after the primer sequences indicate the sequence numbers in Sequence Listing. These primers were used as primers for screening mentioned below.

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Table 2

Gene	aceA	accBC	dtbRI
5'→3'Primer	CCTCTATCCAGCGACTCCG (35)	CATCCACCCCGGTACGGCT (37)	ACGGCCCCAGCCCTGACCGAC (39)
3'→5'Primer	CTGCCCTTGAACCTCACGGTTC (36)	CGGTGACTGGGTGTTCCACC (38)	AGCAGGCCCCATGACGGCGA (40)
PCR conditions for obtaining partial fragment and PCR conditions for screening	94°C, 5 min 98°C, 5 sec 66°C, 2 sec, 30 cycles Z-Taq	94°C, 5 min 98°C, 5 sec 66°C, 2 sec, 30 cycles Z-Taq	94°C, 5 min 98°C, 5 sec 66°C, 2 sec, 30 cycles Z-Taq
Conditions of colony PCR	94°C, 7 min 91°C, 30 sec 55°C, 1 sec 72°C, 2.5 min, 30 cycles Ex-Taq	94°C, 7 min 91°C, 30 sec 55°C, 1 sec 72°C, 2.5 min, 30 cycles Ex-Taq	94°C, 7 min 91°C, 30 sec 55°C, 1 sec 72°C, 2.5 min, 30 cycles Ex-Taq
Amplified fragment	824bp	673bp	805bp

Table 3

Gene	dtSR2	pfk	scrB
5'→3' Primer	ACGGCCAGCCCTGACCGAC (41)	CGTCATCCGAGGAATCGTCC (43)	GGNCGHYTBAAYGAYCC (45)
3'→5' Primer	AGCAGCGCCCTGTGACGGGA (42)	CGTGGGGCCCATGACCTCC (44)	GGRCAYTCCACATRTANCC (46)
PCR conditions for obtaining partial fragment and PCR conditions for screening	94°C, 5 min	94°C, 5 min	94°C, 5 min
	98°C, 5 sec	98°C, 5 sec	98°C, 5 sec
	66°C, 2 sec, 30 cycles	66°C, 2 sec, 30 cycles	50°C, 10 sec
	2-Taq	2-Taq	72°C, 20 sec, 40 cycles 2-Taq
Conditions of colony PCR	94°C, 7 min	94°C 7 min	94°C, 7 min
	91°C, 30 sec	91°C 30 sec	91°C, 30 sec
	55°C, 1 sec	55°C 1 sec	55°C, 1 sec
	72°C, 2.5 min, 30 cycles Ex-Taq	72°C 2.5 min 30 cycles Ex-Taq	72°C, 2.5 min, 30 cycles Ex-Taq
Amplified fragment	805bp	472bp	500bp

Table 4

Gene	<i>gluABCD</i>	<i>pdhA</i>
5'→3'Primer	CCATCCGGATCCGGCAAGTC (47)	ACTGTGTCCATGGGTCTTGGCCC (49)
3'→5'Primer	AATCCCATCTCGTGGGTAAC (48)	CGCTGGAATCCGAACATCGA (50)
PCR conditions for obtaining partial fragment	94°C, 5 min 98°C, 5 sec 50°C, 10 sec 72°C, 20 sec, 30 cycles Z-Taq	94°C, 5 min 98°C, 5 sec 50°C, 10 sec 72°C, 20 sec, 30 cycles Z-Taq
Amplified fragment	500bp	1200bp
Conditions for screening PCR and colony PCR	94°C, 5 min 94°C, 30 sec 50°C, 1 min 72°C, 2 min, 30 cycles EX-Taq	94°C, 5 min 94°C, 30 sec 50°C, 1 min 72°C, 2 min, 30 cycles EX-Taq

Table 5

Gene	<i>pc</i>	<i>ppc</i>
5'→3'Primer	GCGCAACCTACGACGTTGCAATGCG (51)	GGTTCCTGGATTGGTGGAGA(53)
3'→5'Primer	TGGCCGCCTGGGATCTCGTG (52)	CCGCCATCCTTGTGGAATC(54)
PCR conditions for obtaining partial fragment	94°C, 5 min 98°C, 5 sec 55°C, 80 sec 30 cycles Z-Taq	94°C 5 min 98°C 5 sec 50°C 5 sec 72°C 10 sec 30 cycles Z-Taq
Amplified fragment	781bp	1000bp
Conditions for screening PCR	94°C, 5 min 98°C, 5 sec 55°C, 80 sec 30 cycles Z-Taq	94°C, 5 min 98°C, 5 sec 50°C, 5 sec 72°C, 10 sec, 30 cycles Z-Taq
Conditions for colony PCR	94°C, 5 min, 1 cycles 98°C, 5 sec 55°C, 80 sec, 50 cycles Z-Taq	94°C, 5 min 98°C, 5 sec 50°C, 10 sec 72°C, 20 sec, 50 cycles Z-Taq

Table 6

Gene	acn	icd	lpd
5'→3'Primer	GTIGGIACIGAYTCSCATAC (55)	GACATTTCACTCGCTGGACG (57)	ATCATCGCAACCGGTTTC (59)
3'→5'Primer	GCIGGAGAAIATGTGTCIGT (56)	CCGTACTCTTCAGCCCTTCTG (58)	CGTCACCGATGGCGTAAAT (60)
PCR conditions for obtaining partial fragment	94°C, 1 min 96°C, 20 sec 45°C, 1 min 68°C, 2 min, 30 cycles EX-Tag	94°C, 5 min 98°C, 5 sec 55°C, 80 sec, 30 cycles Z-Tag	94°C, 5 min 98°C, 5 sec 50°C, 10 sec 72°C, 20 sec, 30 cycles Z-Tag
Amplified fragment	1500bp	1500bp	500bp
Conditions for screening PCR and colony PCR	Same as above	Same as above	94°C, 5 min 94°C, 30 sec 57°C, 1 min 72°C, 1 min, 30 cycles Ex-Tag
Screening PCR 5'→3'Primer 3'→5'Primer			TACGAGGAGCAGATCCTCAA (63) TTGACGCCGGGTGTTCTCCAG (64)

Table 6 (Cont.)

Gene	acn	lcd	Lpd
LA cloning (N') 3'→5' primer	S1:GGTGAAGCTAAGTAGTTAGC (65) S2:AGCTACTAAACCTGCACC (66)	S1:CCGTACTCTTCAGCCCTTCTG (67) S2:TCGTCCCTTGTCCACATC (68)	S1:ATCATCGCAACCGGTTTC (69) S2:TACGAGGAGCAGATCCTCAA (70)
LA Cloning (C') 5'→3' primer	S1:GCTAACTACTTAGCTTCACC (71) S2:GAACCCAGGAACCTATTGAACC (72)	S1:TCCGATGTCATCATCGAC (73) S2:ATGTGGAACAAGGACGAC (74)	
Restriction enzyme	PstI(N') HindIII(C')	Sali(N') PstI(C')	HindIII
Conditions for LA cloning	N' 94°C, 1 min 94°C, 30 sec 57°C, 2 min 72°C, 2 min, 30 cycles LA-Taq	94°C, 1 min 94°C, 30 sec 57°C, 2 min 72°C, 2.5 min, 30 cycles LA-Taq	94°C, 1 min 94°C, 30 sec 57°C, 2 min 72°C, 1 min, 30 cycles LA-Taq
	C' 94°C, 1 min 94°C, 30 sec 57°C, 2 min 72°C, 2.5 min, 30 cycles LA-Taq		

Table 7

Gene	odhA	
5'→3'Primer	ACACCGTGGTCGCCTCAACG (61)	
3'→5'Primer	TGCTAACCCGTCCCACCTGG (62)	
PCR conditions for obtaining partial fragment	94°C, 5 min 98°C, 5 sec 66°C, 2 sec, 30 cycles Z-Taq	
Amplified fragment	1306bp	
LA cloning (N') 5'→3'Primer	S1:GTACATATTGTCGTTAGAACGCGTAATACGACTCA(75) S2:CGTTAGAACGCGTAATACGACTCACTATAGGGAGA(76)	
Restriction	XbaI	
Conditions for LA cloning	First time	94°C, 30 sec 55°C, 2 min 72°C, 1 min 30 cycles LA-Taq
	Second time	94°C, 1 min 98°C, 20 sec 68°C, 15 min, 30 cycles 72°C 10 min LA-Taq

<3> Screening of plasmid library by PCR

[0097] A clone containing a target gene was selected from the plasmid library by PCR. Sixty colonies were picked up from each plasmid library, and replicated onto two LB agar medium plates. The 60 colonies of each plate were combined, inoculated to a test tube containing 4 ml of LB liquid medium and cultured for 15 hours. Then, a plasmid mixture was respectively obtained by using a plasmid DNA extraction kit produced by Promega. By using this plasmid mixture as a template and primers for screening prepared for each target gene, PCR was performed with the conditions shown as "conditions for screening PCR" in each table to select a clone from which a DNA fragment of the same size as that obtained by PCR using chromosomal DNA as a template had been amplified.

[0098] The nucleotide sequence of the amplified DNA fragment was determined by using a Big Dye dye terminator cycle sequencing kit produced by Perkin-Elmer, and investigating its homology to known gene information to determine if the target gene was obtained or not.

[0099] As for *lpd*, since any DNA fragment was not amplified with the primers produced in <2>, other primers for screening were prepared based on the determined nucleotide sequence.

<4> Selection of clone harboring target gene by colony PCR

[0100] By using a plate that was an origin of a plasmid mixture for which amplification of the target gene fragment was confirmed, colony PCR was performed to select a clone containing the gene fragment. The colony PCR was performed with the conditions shown in Tables 2-7.

[0101] Plasmid DNA was collected from a selected transformant and the nucleotide sequence of the inserted DNA fragment was determined. When the full length of the target gene was not inserted in the inserted DNA fragment, and a upstream region, downstream region or the both were deleted, primers were prepared based on the determined nucleotide sequence, with which a gene fragment comprising the nucleotide sequence of the target gene in its full length was obtained by using TaKaRa LA PCR in vitro Cloning Kit (Takara Shuzo). Then, its nucleotide sequence was determined.

[0102] The outline of LA PCR cloning was as follows. Two kinds of primers each having one of the nucleotide sequences of two regions of the inserted DNA fragment were produced. Chromosomal DNA of *Corynebacterium thermoaminogenes* AJ12310 strain was digested with various restriction enzymes, and ligated to a cassette primer corresponding to each of the restriction enzymes. By using this as a template, PCR was performed with a primer (S1) corresponding to a position distant from the deletion region and a cassette primer (C1) corresponding to a position outside the cassette primer among the prepared primers. Then, another PCR was performed with a primer (S2) corresponding to a position near the deletion region and a cassette primer (C2) corresponding to a position inside the cassette primer among the prepared primers. In this way, a DNA fragment containing the deleted region was obtained. By ligating the obtained DNA fragment with the already obtained DNA fragment, a DNA fragment containing the target gene in full length could be obtained. Since 5' end of the cassette did not have a phosphate group, a nick was formed at the ligation site of the 3' end of the DNA fragment and the 5' end of the cassette. Therefore, the DNA synthesis from the primer C1 stopped at this ligation site in the first PCR, and thus non-specific amplification did not occur. Therefore, specific amplification could be attained.

[0103] The primers and the reaction conditions used for the LA PCR cloning are shown in Tables 2-7. In the tables, the primers mentioned with "(N)" are primers used for the cloning of an upstream deleted portion, and the primers mentioned with "(C)" are primers used for the cloning of a downstream deleted portion. PCR was performed twice according to the instruction attached to the LA PCR cloning kit. Among the primers mentioned in the tables, the primers (S1) used for the first reaction are shown in the upper row, and the primers (S2) used for the second reaction are shown in the lower row.

[0104] The nucleotide sequences of the DNA fragments containing each gene obtained as described above were determined in the same manner as mentioned above. Those nucleotide sequences and amino acid sequences that can be encoded by those nucleotide sequences are shown in SEQ ID NOS: 1-34. The sequences shown with the sequence numbers are summarized in Explanation of Sequence Listing mentioned hereinafter.

[0105] As for *scrB*, any open reading frame was not found. Since the *Corynebacterium thermoaminogenes* AJ12310 strain did not have the invertase activity and did not have sucrose assimilating property, an *scrB* gene fragment was obtained in a similar manner from *Corynebacterium thermoaminogenes* AJ12340 and AJ12309 strains having the sucrose assimilating property. As a result, a DNA fragment having an open reading frame was obtained from the both strains.

Example 2: Acquisition of *gdh* and *gltA* gene

<1> Investigation of GDH activity of *Corynebacterium thermoaminogenes*

[0106] Cells of a wild strain of *Corynebacterium thermoaminogenes*, the AJ12310 strain, was grown on CM-2B agar medium (1 g/dl of yeast extract (produced by Difco), 1 g/dl of polypeptone (produced by Nippon Seiyaku), 0.5 g/dl of NaCl, 10 µg/dl of biotin, 1.5 g/dl of agar, adjusted to pH 7.0 with KOH). The cells were inoculated to a 500-ml volume flask containing 20 ml of a medium for flask having the following composition and cultured at 37°C for 17 hours (until the residual sugar reached about 1 g/dl).

[0107] Similarly, cells of the 2256 strain (ATCC13869) of *Brevibacterium lactofermentum* grown on CM-2B agar medium were cultured at 31.5°C for 17 hours.

[Medium for flask]

[0108]

Glucose	3 g/dl
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(continued)

	KH ₂ PO ₄	0.1 g/dl
	MgSO ₄ ·H ₂ O	0.04 g/dl
5	FeSO ₄ ·7H ₂ O	1 mg/dl
	MnSO ₄ ·4H ₂ O	1 mg/dl
	Vitamin B ₁ -HCl	200 µg/L
	Biotin	50 µg/L
	(NH ₄) ₂ SO ₄	1.5 g/dl
10	Soybean protein hydrolysis solution (Memenō (T-N))	48 mg/dl
	CaCO ₃ (Official reagent) (separately sterilized)	5 g/dl
15	pH 8.0 (adjusted with KOH)	

[0109] About 1 ml of the above culture medium was centrifuged at 1000 rpm for 1 minute to remove CaCO₃, and the cells were washed twice with 200 mM K-phosphate buffer (pH 6.9) and suspended in 300 µl of the same buffer. The obtained cell suspension was sonicated for 5 minutes to disrupt the cells, centrifuged at 1000 rpm for 30 minutes to obtain a crude enzyme solution as the supernatant.

[0110] The optimum reaction temperature and the thermal stability of GDH activity were investigated using the aforementioned crude enzyme solution. The measurement of GDH activity was performed by adding the crude enzyme solution to a reaction mixture (100 mM Tris-HCl (pH 8.0), 20 mM NH₄Cl, 10 mM sodium α-ketoglutarate, 0.25 mM NADPH) and measuring change of absorbance at 340 nm. The protein concentration of the crude enzyme solution was quantified by the Bradford method (Bio-Rad Protein Assay Kit was used) using bovine serum albumin as the standard through measurement of absorbance at 595 nm. The absorbance was measured by using HITACHI U-2000 (produced by Hitachi).

[0111] The GDH activity measured at various reaction temperatures is shown in Fig. 1. While the ATCC13869 strain showed the highest specific activity of GDH around 37°C and the activity markedly decreased around 42°C, the AJ12310 strain showed the highest specific activity around 42°C and it showed the activity even at 45°C.

[0112] Then, the thermal stability of GDH was investigated. The crude enzyme solution was left at 65°C for 0 to 30 minutes before the reaction, and then the enzyme activity was measured at 30°C. The results are shown in Fig. 2. As clearly seen from the results, while GDH of the ATCC13869 strain was inactivated by the heat treatment for 5 minutes, GDH of the AJ12310 strain maintained the activity even after the heat treatment for 30 minutes. In addition, the crude enzyme solution of the AJ12310 strain showed substantially no change in the GDH activity even after the heat treatment at 65°C for 90 minutes (data are not shown).

<2> Examination of CS activity of *Corynebacterium thermoaminogenes*

[0113] The optimum reaction temperature and thermal stability of CS were investigated by using crude enzyme solutions prepared from the cells of the *Corynebacterium thermoaminogenes* AJ12310 strain and the *Brevibacterium lactofermentum* ATCC13869 strain in the same manner as in Example 1. The measurement of CS activity was performed by adding each crude enzyme solution to a reaction mixture (100 mM Tris-HCl (pH 8.0), 0.1 mM DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)), 200 mM sodium L-glutamate, 0.3 mM acetyl CoA), and measuring change of the absorbance at 412 nm.

[0114] The CS activity measured at various reaction temperatures is shown in Fig. 3. The ATCC13869 strain showed the highest specific activity of CS around 23°C and the activity markedly decreased around 33°C. However, the AJ12310 strain showed high specific activity in a reaction temperature-dependent manner up to around 37°C and it showed the activity even at 40°C in a degree corresponding to about 40% of the activity at 37°C.

[0115] Then, thermal stability of CS was investigated. The crude enzyme solution was left at 33-55°C for 5 minutes before the reaction, and then the enzyme activity was measured at 30°C. The results are shown in Fig. 4. Whereas CS of the ATCC13869 strain was inactivated by the heat treatment at 35-40°C, CS of the AJ12310 strain maintained about 40% of the activity even after the heat treatment at 50°C.

<3> Acquisition of *gdh* gene of *Corynebacterium thermoaminogenes*

[0116] The already reported nucleotide sequences of *gdh* gene of various microorganisms were compared. A region

in which nucleotide sequences were well conserved was selected, and primers having the nucleotide sequences shown in SEQ ID NOS: 77 and 78 were prepared based on the nucleotide sequence of the region.

[0117] PCR was performed by using chromosomal DNA prepared from the *Corynebacterium thermoaminogenes* AJ12310 strain using Bacterial Genome DNA Purification Kit (produced by Advanced Genetic Technologies) as a template and the aforementioned primers. Based on the obtained DNA fragment, genome walking was performed by using TaKaRa LA PCR in vitro Cloning Kit (produced by Takara Shuzo) to obtain the whole *gdh* gene, of which whole nucleotide sequence was determined. The result is shown in SEQ ID NO: 79. Further, the amino acid sequence deduced from this nucleotide sequence is shown in SEQ ID NO: 80.

[0118] The *gdh* gene of the *Brevibacterium lactofermentum* ATCC13869 strain was obtained in a similar manner, and its nucleotide sequence was determined. The result is shown in SEQ ID NO: 81. The amino acid sequence encoded by this nucleotide sequence is shown in SEQ ID NO: 82.

[0119] Homology was investigated for the nucleotide sequences of the *gdh* gene and the amino acid sequences of GDH of the *Corynebacterium thermoaminogenes* AJ12310 strain and the *Brevibacterium lactofermentum* ATCC13869 strain determined as described above, and the known *gdh* gene and amino acid sequence of GDH of the *Corynebacterium glutamicum* (C. *glutamicum*) ATCC13032 strain (Molecular Microbiology 6, 317-326 (1992)). The results are shown in Table 8 (for nucleotide sequences) and Table 9 (for amino acid sequences).

Table 8:

Homology of nucleotide sequences of various <i>gdh</i> genes			
	ATCC13869	ATCC13032	AJ12310
ATCC13869	-	94.5%	82.4%
ATCC13032	-	-	78.1%
AJ12310	-	-	-

Table 9:

Homology of amino acid sequences of various GDH			
	ATCC13869	ATCC13032	AJ12310
ATCC13869	-	90.8%	91.7%
ATCC13032	-	-	83.4%
AJ12310	-	-	-

<4> Acquisition of *gltA* gene of *Corynebacterium thermoaminogenes*

[0120] The already reported nucleotide sequences of *gltA* gene of various microorganisms were compared. A region in which nucleotide sequences were well conserved was selected, and primers having the nucleotide sequences shown in SEQ ID NOS: 83 and 84 were prepared based on the nucleotide sequence of the region.

[0121] PCR was performed by using chromosomal DNA prepared from the *Corynebacterium thermoaminogenes* AJ12310 strain (FERM BP-1542) using Bacterial Genome DNA Purification Kit (produced by Advanced Genetic Technologies) as a template and the aforementioned primers 7 and 8, and the nucleotide sequence of the amplified nucleotide sequence of about 0.9 kb was determined.

[0122] On the basis of the obtained nucleotide sequence of *gltA* gene of *Corynebacterium glutamicum* (Microbiol., 140, 1817-1828 (1994)), the primers of SEQ ID NOS: 85, 86, 87 and 88 were prepared. PCR was performed in a manner similar to the above by using chromosomal DNA of AJ12310 as a template and the primers of SEQ ID NOS: 85, 86, 87 and 88, and the nucleotide sequence of the amplified DNA fragment was specified to determine the whole nucleotide sequence of the *gltA* gene. The result is shown in SEQ ID NO: 89. Further, an amino acid sequence expected from this nucleotide sequence is shown in SEQ ID NO: 90.

[0123] The *gltA* gene of the *Brevibacterium lactofermentum* 2256 strain was obtained in a similar manner, and its nucleotide sequence was determined. The result is shown in SEQ ID NO: 91. The amino acid sequence encoded by this nucleotide sequence is shown in SEQ ID NO: 92.

[0124] Homology was investigated for the nucleotide sequences of the *gltA* gene and the amino acid sequences of CS of the *Corynebacterium thermoaminogenes* AJ12310 strain and the *Brevibacterium lactofermentum* ATCC13032 strain determined as described above, and the known *gltA* gene and amino acid sequence of CS of the *Corynebacterium*

glutamicum ATCC13032 strain (*Microbiol.* 140, 1817-1828 (1994)): The results are shown in Table 10 (for nucleotide sequences) and Table 11 (for amino acid sequences).

Table 10:

Homology of nucleotide sequences of various <i>gltA</i> genes			
	ATCC13869	ATCC13032	AJ12310
ATCC13869	-	99.5%	85.7%
ATCC13032	-	-	85.6%
AJ12310	-	-	-

Table 11:

Homology of amino acid sequences of various CS			
	ATCC13869	ATCC13032	AJ12310
ATCC13869	-	99.3%	92.1%
ATCC13032	-	-	92.1%
AJ12310	-	-	-

Example 3: Acquisition of *scrB* gene of *Corynebacterium thermoaminogenes*

[0125] Since an *scrB* gene fragment was obtained from the *Corynebacterium thermoaminogenes* AJ12309 strain as shown in Example 1, it was attempted to obtain the total sequence of the gene. First, a partial fragment was obtained in the same manner as in Example 1 using the primers shown in SEQ ID NO: 45 and SEQ ID NO: 46. These primers were synthesized based on the *scrB* sequence of the *Brevibacterium lactofermentum* 2256 strain (Japanese Patent Laid-open No. 08-196280/1996).

[0126] Separately, chromosomal DNA was prepared from the AJ12309 strain by using Bacterial Genome DNA Purification Kit (Advanced Genetic Technologies Corp.). Sterilized water was added to 0.5 µg of this chromosomal DNA, 50 pmol each of the aforementioned primers, 4 µl of dNTP mixture (2.5 mM each), 5 µl of 10 x Z-Taq Buffer (Takara Shuzo) and 2 U of Z-Taq (Takara Shuzo) to prepare a PCR reaction mixture in a total volume of 50 µl. PCR was performed with a cycle of denaturation at 98°C for 5 seconds, association at 50°C for 10 seconds and extension reaction at 72°C for 20 seconds, which was repeated for 30 cycles, by using the above reaction mixture and a thermal cycler GeneAmp PCR System 9600 (PE) to amplify a partial fragment of *scrB* of about 600 bp.

[0127] Then, the total sequence of *scrB* was determined by using an LA PCR in vitro Cloning Kit (Takara Shuzo). All of the procedure was performed in accordance with the protocol attached to the LA PCR in vitro Cloning Kit. Based on the obtained partial sequence, primers shown in SEQ ID NOS: 97, 98, 99 and 100 were synthesized. For the first PCR reaction for sequencing an upstream region, the primers shown in SEQ ID NOS: 95 and 97 and chromosomal DNA of AJ12309 strain digested with *EcoT14I* as a template DNA were used. For the second PCR reaction, the primers shown in SEQ ID NOS: 96 and 98 were used. For the first PCR reaction for sequencing a downstream region, the primers shown in SEQ ID NOS: 95 and 99 and chromosomal DNA of AJ12309 strain digested with *SalI* (Takara Shuzo) as a template DNA were used. For the second PCR reaction, the primers shown in SEQ ID NOS: 96 and 100 were used. By the above procedure, a sequence of a full length of 1656 bp containing ORF of *scrB* was determined. This nucleotide sequence is shown in SEQ ID NO: 93, and a deduced amino acid sequence is shown in SEQ ID NO: 94.

Example 4: Examination of thermal stability of isocitrate lyase, phosphofructokinase, phosphoenolpyruvate carboxylase, aconitase, isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase

[0128] Thermal stability was investigated for the following enzymes derived from *Corynebacterium thermoaminogenes*. In this Example, protein concentrations were measured by the Bradford method (Bio-Rad Protein Assay Kit was used) using bovine serum albumin as a standard protein. Further, measurement of absorbance was performed by using HITACHI U-2000 (Hitachi) unless otherwise indicated.

<1> Isocitrate lyase

[0129] Thermal stability of activity of isocitrate lyase (henceforth also referred to as "ICL") derived from the *Corynebacterium thermoaminogenes* AJ12310 strain and ICL derived from the *Brevibacterium lactofermentum* 2256 strain (ATCC13869) was investigated. For the activity measurement, used were cells of which culture in a medium having the composition mentioned in Table 2 was terminated before all of the carbon source was completely consumed. The method of the activity measurement was one described in Dieter J. Reinscheid *et al.*, *J. Bacteriol.*, 176 (12), 3474 (1994). Specifically, the cells were washed with 50 mM Tris buffer (pH 7.3), suspended in the same buffer, and disrupted by sonication (INSONATOR 201M produced by KUBOTA was used, 200 W, 5 minutes). After the sonication, the suspension was centrifuged (13000 x g, 30 minutes) to remove undisrupted cells to prepare a crude enzyme solution.

[0130] The crude enzyme solution was added to a reaction system containing 50 mM MOPS-NaOH (pH 7.3), 5 mM dithiothreitol, 15 mM MgCl₂, 1 mM EDTA, 5 mM D-threo-isocitrate, 0.2 mM NADH and 18 U of LDH (lactate dehydrogenase), and absorbance at 340 nm at various temperatures (30, 40, 50, 60 or 70°C) was measured by a Hitachi spectrophotometer U-3210. The measurement results for various reaction temperatures were shown in Fig. 5. Further, the crude enzyme solution was pretreated at 50°C (pretreatment time: 5 minutes or 15 minutes), and the activity was measured at 37°C. The results are shown in Fig. 6.

[0131] As a result, ICL of the AJ12310 strain showed the maximum activity at 60°C, whereas ICL of the 2256 strain showed the maximum activity around 50°C. Further, while ICL of the 2256 strain was completely inactivated after the pretreatment for 5 minutes, ICL of the AJ12310 strain maintained half of the activity after the pretreatment for 5 minutes. Thus, the stability of ICL of the AJ12310 strain at high temperatures was confirmed.

Table 12

Composition of medium for ICL activity measurement	
Component	Concentration
(NH ₄) ₂ SO ₄	5 g/l
Urea	5 g/l
KH ₂ PO ₄	0.5 g/l
K ₂ HPO ₄	0.5 g/l
MOPS	20.9 g/l
MgSO ₄ ·7H ₂ O	0.25 g/l
CaCl ₂ ·7H ₂ O	10 mM
CuSO ₄ ·7H ₂ O	0.2 mg/l
Biotin	0.2 mg/l
MnSO ₄ ·7H ₂ O	10 mg/l
FeSO ₄ ·7H ₂ O	10 mg/l
ZnSO ₄ ·7H ₂ O	1 mg/l
Acetic acid	4%

<2> Phosphofructokinase

[0132] Thermal stability of activity of phosphofructokinase (henceforth also referred to as "PKF") derived from the *Corynebacterium thermoaminogenes* AJ12310 strain and PKF derived from the *Brevibacterium lactofermentum* 2256 strain was investigated. For the activity measurement, used were cells of which culture in a medium having the composition mentioned in Table 13 was terminated before all of the saccharide was completely consumed. The method of the activity measurement was one described in Michiko Mori *et al.*, *Agric. Biol. Chem.*, 51 (10), 2671 (1994). Specifically, the cells were washed with 0.1 M Tris buffer (pH 7.5), suspended in the same buffer, and disrupted by sonication (INSONATOR 201M produced by KUBOTA was used, 200 W, 5 minutes). After the sonication, the suspension was centrifuged (13000 x g, 30 minutes) to remove undisrupted cells to obtain a crude enzyme solution.

[0133] The crude enzyme solution was added to a reaction system containing 100 mM Tris buffer (pH 7.5), 0.2 mM NADH, 10 mM MgCl₂, 2 mM NH₄Cl, 10 mM KCl, 0.2 mM phosphoenolpyruvic acid, 6.4 mM fructose-6-phosphate, 1 mM ATP and 40 µg of LDH/PK (pyruvate kinase), and absorbance at 340 nm was measured at various temperatures

(30, 40, 50, 60 or 70°C) by a Hitachi spectrophotometer U-3210. The measurement results for various reaction temperatures were shown in Fig. 7. Further, the crude enzyme solution was pretreated at 50°C (pretreatment time: 1, 3, 5 or 10 minutes), and the activity was measured at 37°C. The results are shown in Fig. 8.

[0134] As a result, PKF of the AJ12310 strain showed the maximum activity around 50°C, whereas PKF of the 2256 strain showed the maximum activity around 30°C. Thus, it was confirmed that the optimum temperature of PKF of the AJ12310 strain resided in a high temperature region.

Table 13

Composition of medium for PFK activity measurement	
Component	Concentration
Polypeptone	20 g/l
Yeast extract	20 g/l
Sodium chloride	5 g/l
Glucose	20 g/l

<3> Phosphoenolpyruvate carboxylase

[0135] Thermal stability of activity of phosphoenolpyruvate carboxylase (henceforth also referred to as "PEPC") derived from the *Corynebacterium thermoaminogenes* AJ12310 strain and PEPC of the *Brevibacterium lactofermentum* 2256 strain was examined.

[0136] Cells of the AJ12310 strain grown on CM-2B agar medium were inoculated to a 500-ml volume flask containing 20 ml of a medium for flask (8 g/dl of Glucose, 0.1 g/dl of KH_2PO_4 , 0.04 g/dl of $\text{MgSO}_4 \cdot \text{H}_2\text{O}$, 1 mg/dl of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 5 mg/dl of $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 3 g/dl of $(\text{NH}_4)_2\text{SO}_4$, 48 mg/dl of TN (soybean protein hydrolysis solution), 200 µg/L of vitamin B₁, 300 µg/L of biotin, 50 µl/l of GD-113 (antifoaming agent), 5 g/dl of CaCO_3 (Official reagent, separately sterilized), pH 8.0 (adjusted with KOH)), and cultured at 37°C. Cells of the 2256 strain grown on CM-2B agar medium were similarly cultured at 31.5°C.

[0137] The above culture broth in which the cells were grown to the logarithmic growth phase was centrifuged at 1000 rpm for 1 minute to remove CaCO_3 , and the cells were washed 3 times with washing buffer (100 mM Tris/HCl pH 8.0, 10 mM MgSO_4 , 1 mM DTT, 20% glycerol), sonicated to disrupt the cells, and centrifuged at 15 krpm for 10 minutes to remove cell debris. The supernatant was further centrifuged at 60 krpm for 1 hour to obtain a crude enzyme solution as the supernatant.

[0138] By using the above crude enzyme solution, optimum reaction temperature and thermal stability of the PEPC activity were investigated. The measurement of PEPC activity was performed by adding the crude enzyme solution to a reaction mixture (100 mM Tris/ H_2SO_4 (pH 8.5), 5 mM phosphoenolpyruvic acid, 10 mM KHCO_3 , 0.1 mM acetyl-CoA, 0.15 mM NADH, 10 mM MgSO_4 , 10 U of malate dehydrogenase, 0.1 mM DTT), and measuring change of the absorbance at 340 nm in 800 µl of reaction volume.

[0139] The PEPC activity measured at various reaction temperatures is shown in Fig. 9. While the activity of the 2256 strain markedly decreased at 40°C, the AJ12310 strain showed substantially no decrease of the activity even at 40°C.

[0140] Then, the thermal stability of PEPC was investigated. The crude enzyme solution was left at 45°C for 0-20 minutes before the reaction, and then the enzyme activity was measured at 20°C. The results are shown in Fig. 10. As clearly seen from the results, whereas the PEPC activity of the 2256 strain was substantially lost after the heat treatment for 10 minutes, PEPC of the AJ12310 strain maintained the activity even after the heat treatment for 20 minutes.

[0141] These results demonstrated the stability of PEPC of the AJ12310 strain at a high temperature.

<4> Aconitase

[0142] Aconitase (henceforth also referred to as "ACN") derived from the *Corynebacterium thermoaminogenes* AJ12310 strain and ACN derived from the *Brevibacterium lactofermentum* 2256 strain were measured, and thermal stability thereof was examined.

[0143] Cells of the AJ12310 strain grown on CM-2B agar medium were inoculated to a 500-ml volume flask containing 20 ml of a medium for flask having the same composition as mentioned in <3>, and cultured at 37°C. Cells of the 2256 strain grown on CM-2B agar medium were similarly cultured at 31.5°C.

[0144] The above culture broth in which the cells were grown to the logarithmic growth phase was centrifuged at

1000 rpm for 1 minute to remove CaCO_3 , and the cells were washed 3 times with 50 mM Tris/HCl pH 7.5, sonicated to disrupt the cells, and centrifuged at 15 krpm for 10 minutes to obtain a crude enzyme solution as the supernatant.

[0145] By using the above crude enzyme solution, optimum reaction temperature and thermal stability of ACN activity were investigated. The measurement of ACN activity was performed by adding the crude enzyme solution to a reaction mixture (20 mM Tris/HCl (pH 7.5), 50 mM NaCl, 20 mM isocitrate-3Na), and measuring change of the absorbance at 240 nm in 800 μl of reaction volume.

[0146] The ACN activity measured at various reaction temperatures is shown in Fig. 11. The AJ12310 strain showed higher activity at a higher temperature compared with the 2256 strain.

[0147] Then, the thermal stability of ACN was investigated. The crude enzyme solution was left at 50°C for 0-15 minutes before the reaction, and then the enzyme activity was measured at 30°C. The results are shown in Fig. 12. As clearly seen from the results, ACN of the AJ12310 strain showed less activity decrease due to the heat treatment compared with ACN of the 2256 strain.

[0148] These results demonstrated the stability of ACN of the AJ12310 strain at a high temperature.

<5> Isocitrate dehydrogenase

[0149] Thermal stability of activity of isocitrate dehydrogenase (henceforth also referred to as "ICDH") derived from the *Corynebacterium thermoaminogenes* AJ12310 strain and ICDH derived from the *Brevibacterium lactofermentum* 2256 strain was examined.

[0150] Cells of the AJ12310 strain grown on CM-2B agar medium were inoculated to a 500-ml volume flask containing 20 ml of a medium for flask having the same composition as mentioned in <3>, and cultured at 37°C. Cells of the 2256 strain grown on CM-2B agar medium were similarly cultured at 31.5°C.

[0151] The above culture broth in which the cells were grown to the logarithmic growth phase was centrifuged at 1000 rpm for 1 minute to remove CaCO_3 , and the cells were washed 3 times with 50 mM Tris/HCl pH 7.5, sonicated to disrupt the cells, and centrifuged at 15 krpm for 10 minutes to obtain a crude enzyme solution as the supernatant.

[0152] By using the above crude enzyme solution, optimum reaction temperature and thermal stability of ICDH activity were investigated. The measurement of ICDH activity was performed by adding the crude enzyme solution to a reaction mixture (35 mM Tris/HCl, 0.35 mM EDTA (pH 7.5), 1.5 mM MnSO_4 , 0.1 mM NADP, 1.3 mM isocitrate-3Na), and measuring change of the absorbance at 340 nm in 800 μl of reaction volume.

[0153] The ICDH activity measured at various reaction temperatures is shown in Fig. 13. While the activity of the 2256 strain markedly decreased at 70°C, substantially no activity decrease was observed even at 70°C for the AJ12310 strain.

[0154] Then, the thermal stability of ICDH was investigated. The crude enzyme solution was left at 45°C for 0-15 minutes before the reaction, and then the enzyme activity was measured at 30°C. The results are shown in Fig. 14. As clearly seen from the results, while only about 15% of residual activity was observed after the heat treatment for 15 minutes for the 2256 strain, about 60% of residual ICDH activity was observed for the AJ12310 strain.

[0155] These results demonstrated the stability of ICDH of the AJ12310 strain at a high temperature.

<6> 2-Oxoglutarate dehydrogenase

[0156] 2-Oxoglutarate dehydrogenase (henceforth also referred to as "ODHC") derived from the *Corynebacterium thermoaminogenes* AJ12310 strain and ODHC derived from the *Brevibacterium lactofermentum* 2256 strain were measured, and thermal stability thereof was examined.

[0157] For the activity measurement, used were cells of which culture in a medium having the composition mentioned in Table 14 was terminated before all of the saccharide was completely consumed. The method of the activity measurement was one described in Isamu Shio et al., Agric. Biol. Chem., 44 (8), 1897 (1980). Specifically, the cells were washed with 0.2% potassium chloride, suspended in 100 mM TES-NaOH (pH 7.5), 30% glycerol solution, and disrupted by sonication (INSONATOR 201M produced by KUBOTA was used, 200 W, 5 minutes). After the disruption by sonication, the suspension was centrifuged (13000 x g, 30 minutes) to remove undisrupted cells, and subjected to gel filtration using the same buffer and Sephadex-G25 to prepare a crude enzyme solution.

[0158] The crude enzyme solution was added to a reaction system containing 100 mM TES-NaOH (pH 7.7), 5 mM MgCl_2 , 0.2 mM Coenzyme A, 0.3 mM cocarboxylase, 1 mM α -ketoglutaric acid, 3 mM L-cysteine and 1 mM acetylpyridine-adenine dinucleotide, and absorbance at 365 nm was measured at various temperatures (30, 40, 50, 60 or 70°C) by a Hitachi spectrophotometer U-3210. The crude enzyme solution was pretreated at 50°C (pretreatment time: 1, 3, 5 or 10 minutes), and the activity was measured at 37°C. The results are shown in Fig. 15.

[0159] As a result, while ODHC of the 2256 strain was completely inactivated by the pretreatment for 10 minutes, ODHC of the AJ12310 strain showed substantially constant activity irrespective of the pretreatment time, and thus its stability against high temperature treatment was confirmed.

Table 14

Composition of medium for ODHC activity measurement	
Component	Concentration
Glucose	80 g/l
KH ₂ PO ₄	1 g/l
MgSO ₄ ·7H ₂ O	0.4 g/l
FeSO ₄ ·7H ₂ O	0.01 g/l
MnSO ₄ ·7H ₂ O	0.05 g/l
(NH ₄) ₂ SO ₄	30 g/l
rotein Soybean protein hydrolysate	480 mg/l
Thiamin hydrochloride	200 µg/l
Biotin	300 µg/l

Example 5: Impartation of sucrose assimilating ability by gene transfer of *scrB* gene

[0160] Since the *Corynebacterium thermoaminogenes* AJ12310 strain did not have invertase activity and sucrose assimilating property, it was investigated if sucrose assimilating ability could be imparted to it by transferring the *scrB* gene of the AJ12309 strain to the strain.

<1> Production of plasmid carrying *scrB* derived from *Corynebacterium thermoaminogenes* AJ12309 strain

[0161] To obtain an *scrB* gene fragment, the primers shown in SEQ ID NOS: 101 and 102 were synthesized, of which both ends were ligated with *Sma*I sequences, based on the nucleotide sequence shown in SEQ ID NO: 93. Sterilized water was added to 0.5 µg of chromosomal DNA of the 12309 strain, 50 pmol each of the aforementioned oligonucleotides, 4 µl of dNTP mixture (2.5 mM each), 5 µl of 10 x Pyrobest Buffer (Takara Shuzo) and 2 U of Pyrobest polymerase (Takara Shuzo) to prepare a PCR reaction mixture in a total volume of 50 µl. PCR was performed with a cycle of denaturation at 98°C for 10 seconds, association at 55°C for 30 seconds and extension reaction at 72°C for 2 minutes, which was repeated for 30 cycles, by using the above reaction mixture and a thermal cycler GeneAmp PCR System 9600 (PE) to amplify a fragment of about 1.7 kb containing *scrB* ORF.

[0162] Then, the above amplified fragment was digested with *Sma*I (Takara Shuzo), and ligated to plasmid pSAC4 containing a dephosphorylated replication origin functioning in coryneform bacteria, which had been digested with *Sma*I, to prepare pSCR155. The construction of pSCR155 is shown in Fig. 16. pSAC4 was produced as follows. In order to make the vector for *Escherichia coli* pHSG399 (Takara Shuzo) autonomously replicable in coryneform bacteria, the replication origin (Japanese Patent Laid-open No. 5-7491/1993) derived from the already obtained plasmid pHM1519 autonomously replicable in coryneform bacteria (Miwa, K. et al., Agric. Biol. Chem., 48 (1984) 2901-2903) was introduced into it. Specifically, pHM1519 was digested with restriction enzymes *Bam*HI and *Kpn*I, and the obtained fragment containing the replication origin was blunt-ended by using a Blunting kit produced by Takara Shuzo and inserted into pHSG399 at the *Sa*I site by using an *Sa*I linker (produced by Takara Shuzo) to obtain pSAC4.

<2> Transfer of plasmid carrying *scrB* gene into AJ12310 strain

[0163] pSCR155 produced above and plasmid pSSM30BS (Japanese Patent Laid-open No. 08-196280/1996) carrying the *scrB* gene derived from *Brevibacterium lactofermentum* were introduced into the *Corynebacterium thermoaminogenes* AJ12310 strain. The transformation was performed according to the following procedure. The cells were inoculated to CM-2B medium containing 20% sucrose in such an amount that OD₆₆₀ of the medium should become 0.1, and cultured at 37°C with shaking until the OD₆₆₀ become 0.3. Lysozyme was added to the medium at a concentration of 100 µg/ml, and the cells were further cultured for 2 hours. The cells were washed three times with 20% sucrose, suspended in 20% sucrose, added with the plasmid collected from *Escherichia coli* JM110, mixed sufficiently, and applied with an electric pulse (18 kV/cm, 300 msec) to be introduced with the DNA. After the cells were subjected to restoration culture overnight in CM-2B medium containing 20% sucrose, transformants were selected on CM-2B agar medium containing 5 µg/ml of chloramphenicol. Specifically, the transformation was performed by the electric pulse method (Japanese Patent Laid-open No. 12-204236/2000, and the selection of transformants was performed

on CM2B plate medium containing 5 µg/ml of chloramphenicol at 37°C. As a result, any transformant harboring the plasmid pSSM30BS carrying *scrB* derived from *Brevibacterium lactofermentum* was not obtained, but only a transformant harboring the plasmid pSCR155 carrying *scrB* derived from *Corynebacterium thermoaminogenes* was obtained. This strain was designated as AJ12310/pSCR155.

<3> Evaluation of culture of AJ12310/pSCR155 strain using sucrose as sugar source.

[0164] AJ12310/pSCR155 prepared above was inoculated to a medium having the composition shown in Table 15, and cultured at 37°C for 22 hours with shaking. The absorbance (OD) and residual sugar (RS) of the medium were measured after the culture. The results are shown in Table 16. As a result, it was confirmed that, while the AJ12310 strain could not assimilate sucrose and hence could not grow, the *scrB* gene introduced strain, the AJ12310/pSCR155 strain, became to be able to assimilate sucrose.

Table 15

Medium composition	
Medium composition	Concentration
Sucrose	60 g/l
KH ₂ PO ₄	1 g/l
MgSO ₄ ·7H ₂ O	0.4 g/l
FeSO ₄ ·7H ₂ O	0.01 g/l
MnSO ₄ ·7H ₂ O	0.01 g/l
(NH ₄) ₂ SO ₄	30 g/l
Soybean protein hydrolysate	480 mg/l
Thiamin hydrochloride	200 µg/l
Biotin	300 µg/l

Table 16

Result of sucrose culture		
	OD (x 51)	RS (g/l)
2256	1.292	0.00
AJ12310	0.058	60.00
AJ12310/pSCR155	1.571	0.84

Example 6: L-glutamic acid production by *pdhA* gene-amplified strain

<1> Construction of plasmid pPDHA-2 carrying *pdhA*

[0165] The *pdhA* gene derived from the *Corynebacterium thermoaminogenes* AJ12310 strain was obtained by screening of a plasmid library. Specifically, PCR was performed with the conditions shown in Example 1, Table 4, using a plasmid library mixture as a template, and a clone p21A was selected, from which a DNA fragment of the same size is amplified as obtained in PCR using chromosomal DNA as a template. The DNA sequence of this plasmid was determined to confirm that the full length of *pdhA* was contained in it.

[0166] p21A was digested with *Xba*I and *Kpn*I to excise a DNA fragment of 4 kb containing the full length of the *pdhA* gene and a promoter region. This DNA fragment containing the *pdhA* gene was inserted into the *Xba*I and *Kpn*I sites of pHSG299 (Takara Shuzo). Then, this plasmid was digested with *Xba*I, and a fragment obtained by digesting pXK4 with *Xba*I was inserted to prepare pPDHA-2. The construction process of pPDHA-2 is shown in Fig. 17. A DNA Ligation Kit Ver.2 (Takara Shuzo) was used for the ligation reaction, and *Escherichia coli* JM109 strain (Takara Shuzo) was used as the host of genetic manipulation. The aforementioned pXK4 was produced as follows. A shuttle vector pHK4 for coryneform bacteria and *Escherichia coli* (Japanese Patent Laid-open No. 5-7491/1993) was digested with restriction enzymes *Bam*HI and *Kpn*I to obtain a DNA fragment containing the replication origin, and the obtained fragment

was blunt-ended by using a DNA blunting kit (Blunting Kit produced by Takara Shuzo), ligated to an *Xba*I linker (produced by Takara Shuzo) and inserted into pHSG299 at the *Xba*I site to obtain the plasmid pKX4.

<2> Transfer of plasmid carrying *pdhA* gene into AJ12310 strain

[0167] The plasmid pPDHA-2 produced above was introduced into the *Corynebacterium thermoaminogenes* AJ12310 strain to prepare a *pdhA* gene-amplified strain. The transformation was performed in the same manner as Example 5, and a transformant was selected on CM-2B agar medium containing 25 µg/ml kanamycin to obtain AJ12310/pPDHA-2 strain.

<3> L-glutamic acid production by *pdhA*-amplified strain

[0168] The AJ12310 strain and the *pdhA* gene-amplified strain obtained above, AJ12310/pPDHA-2 strain, both of which were grown on CM-2B agar medium, were each inoculated to a 500-ml volume flask containing 20 ml of a medium for seed culture flask shown in Table 17, and cultured at 37°C with shaking until glucose was completely consumed. 2 ml of this culture broth was inoculated into 500 ml-volume flask containing 20 ml of a medium for main culture flask shown in Table 17, and cultured as main culture at 37°C and 44°C. The main culture was continued until glucose was completely consumed. After the culture, OD₆₂₀ of the medium and accumulated amount of L-glutamic acid were measured to examine the effect of the gene amplification on the cell formation and production of glutamic acid. The measurement of OD was performed by using a spectrophotometer HITACHI U-2000 (Hitachi), and L-glutamic acid concentration was measured by using a glutamic acid analyzer AS-210 (Asahi Chemical Industry). The results are shown in Fig. 18.

[0169] The *pdhA* gene-amplified strain, AJ12310/pPDHA-2 strain, showed increased L-glutamic acid accumulation and increased OD compared with the AJ12310 strain, and thus it became clear that the amplification of the *pdhA* gene was effective for L-glutamic acid production.

Table 17

Medium for evaluation of <i>pdhA</i> -amplified strain		
Medium composition	Seed culture	Main culture
Sucrose	30 g/l	60 g/l
KH ₂ PO ₄	1 g/l	1 g/l
MgSO ₄ ·7H ₂ O	0.4 g/l	0.4 g/l
FeSO ₄ ·7H ₂ O	0.01 g/l	0.01 g/l
MnSO ₄ ·7H ₂ O	0.01 g/l	0.01 g/l
(NH ₄) ₂ SO ₄	15 g/l	30 g/l
Soybean protein hydrolysate	480 mg/l	480 mg/l
Thiamin hydrochloride	200 µg/l	200 µg/l
Biotin	10 µg/l	
AZ-20R (anti-foaming agent)	20 µg/l	20 µg/l
CaCO ₃ (separately sterilized)	50 g/L	50 g/L
pH 8.0 (adjusted with KOH)		

Example 7: L-glutamic acid production by *icd* gene-amplified strain

<1> Construction of plasmid pICD-4 carrying *icd* derived from *Corynebacterium thermoaminogenes* AJ12310 strain

[0170] Based on the *icd* gene sequence of the AJ12310 strain shown in SEQ ID NO: 29, the primers shown in SEQ ID NO: 103 and SEQ ID NO: 104 were synthesized. A *Bgl*II site was introduced into 5' end of the both primers. Separately, genomic DNA of the *Corynebacterium thermoaminogenes* AJ12310 strain was prepared by using a Genomic DNA Purif. Kit (Edge BioSystems). Sterilized water was added to the genome DNA as a template, 100 pmol each of the aforementioned primers, 8 µl of dNTP mixture (2.5 mM each), 10 µl of 10 x Pyrobest Buffer II (Takara Shuzo) and

2.5 U of Pyrobest polymerase (Takara Shuzo) to prepare a PCR reaction mixture in a total volume of 100 μ l. PCR was performed with a cycle of denaturation at 98°C for 10 seconds, association at 55°C for 1 minute and extension reaction at 72°C for 4 minutes, which was repeated for 30 cycles, by using the above reaction mixture and a thermal cycler TP240 (Takara Shuzo) to amplify a DNA fragment of about 3.3 kb containing the *icd* gene and its promoter.

[0171] Then, this DNA fragment containing the *icd* gene was digested with *Bgl*II, and ligated to pHSG299 (Takara Shuzo) at the *Bam*HI site. This plasmid was then treated with *Xba*I, and a fragment obtained by digesting pXK4 with *Xba*I was inserted into the plasmid to construct pICD-4. The construction procedure of pICD-4 is shown in Fig. 19. A DNA Ligation Kit Ver.2 (Takara Shuzo) was used for the ligation reaction, and *Escherichia coli* JM109 strain (Takara Shuzo) was used as the host of genetic manipulation.

<2> Transfer of plasmid carrying *icd* gene into AJ12310 strain

[0172] The plasmid pICD-4 produced above was introduced into the *Corynebacterium thermoaminogenes* AJ12310 strain to prepare an *icd* gene-amplified strain. The transformation was performed in the same manner as Example 5, and a transformant was selected on CM-2B agar medium containing 25 μ g/ml kanamycin to obtain AJ12310/pICD-4 strain.

<3> L-glutamic acid production by *icd*-amplified strain

[0173] Culture evaluation was performed for the AJ12310 strain and the *icd*-amplified strain thereof, AJ12310/pICD, by the culture method described in Example 6. The results are shown in Fig. 20. The *icd* gene-amplified strain AJ12310/pICD-4 strain showed increased L-glutamic acid accumulation and increased OD compared with the AJ12310 strain, and thus it became clear that the amplification of the *icd* gene was effective for L-glutamic acid production.

Example 8: L-glutamic acid production by *gdh* gene-amplified strain

<1> Construction of plasmid carrying *gdh* derived from *Corynebacterium thermoaminogenes* AJ12310 strain

[0174] Based on the *gdh* gene sequence of the AJ12310 strain shown in SEQ ID NO: 79, the primers shown in SEQ ID NO: 105 and SEQ ID NO: 106 were synthesized.

[0175] Separately, chromosomal DNA of the AJ12310 strain was prepared by using a Bacterial Genome DNA Purification Kit (Advanced Genetic Technologies Corp.). Sterilized water was added to 0.5 μ g of this chromosomal DNA, 10 pmol each of the aforementioned oligonucleotides, 8 μ l of dNTP mixture (2.5 mM each), 5 μ l of 10 x LA Taq Buffer (Takara Shuzo) and 2 U of LA Taq (Takara Shuzo) to prepare a PCR reaction mixture in a total volume of 50 μ l. PCR was performed with a cycle of denaturation at 94°C for 30 seconds, association at 55°C for 1 second and extension reaction at 72°C for 3 minutes, which was repeated for 30 cycles, by using the above reaction mixture and a thermal cycler TP240 (Takara Shuzo) to amplify a DNA fragment of about 2 kb containing the *gdh* gene and its promoter. The obtained amplified fragment was digested with *Pst*I (Takara Shuzo), mixed with pHSG299 (Takara Shuzo) fully digested with *Pst*I and ligated to it. A DNA Ligation Kit Ver.2 produced by Takara Shuzo was used for the ligation reaction. After the ligation, competent cells of *Escherichia coli* JM109 (produced by Takara Shuzo) were transformed with the ligation product, plated on L medium (10 g/l of Bacto-trypton, 5 g/l of Bacto-yeast extract, 5 g/l of NaCl, 15 g/l of agar, pH 7.2) containing 10 μ g/ml of IPTG (isopropyl- β -D-thiogalactopyranoside), 40 μ g/ml of X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactoside) and 40 μ g/ml of chloramphenicol, and cultured overnight. The emerged white colonies were picked up and subjected to single colony separation to obtain transformants.

[0176] Plasmids were prepared from the transformants by the alkali method (Text for Bioengineering Experiments, Edited by the Society for Bioscience and Bioengineering, Japan, p.105, Baifukan, 1992) and their restriction maps were prepared. A plasmid having a restriction map equivalent to that shown in Fig. 21 was designated as pHSG299YGDH.

[0177] A replication origin that functions in coryneform bacteria was introduced into this pHSG299YGDH. Specifically, pXC4 was digested with a restriction enzyme *Xba*I to obtain a fragment containing a replication origin derived from pHM1519, and it was mixed with pHSG299YGDH fully digested with *Xba*I and ligated to it. Plasmids were prepared in the same manner as above and a plasmid having a restriction map equivalent to that shown in Fig. 21 was designated as pYGDH. pXC4 was constructed in the same manner as that for pXK4 mentioned in Example 6 except that pHSG399 (Cm^r) was used instead of pHSG299.

<2> Transfer of plasmid carrying *gdh* gene into AJ12310

[0178] The plasmid produced above was introduced into the *Corynebacterium thermoaminogenes* AJ12310 strain

to prepare a *gdh* gene-amplified strain. The transformation was performed in the same manner as Example 5, and a transformant was selected on CM-2B agar medium containing 25 µg/ml kanamycin at 31°C to obtain AJ12310/pYGDH.

<3> L-glutamic acid production by *gdh*-amplified strain

[0179] The AJ12310 strain and the *gdh* gene-amplified strain obtained above, AJ12310/pYGDH strain, both of which were grown on CM-2B agar medium, were each inoculated to a 500-ml volume flask containing 20 ml of a medium for seed culture flask shown in Table 18, and cultured at 37°C with shaking until glucose was completely consumed. 2 ml of this culture broth was inoculated into 500 ml-volume flask containing 20 ml of a medium for main culture flask shown in Table 19, and cultured as main culture at 37°C and 44°C. The main culture was continued until glucose was completely consumed. After completion of the culture, OD₆₂₀ of the medium and accumulated amount of L-glutamic acid were measured to examine the effect of the gene amplification on the cell formation and production of glutamic acid. The measurement of OD was performed by using a spectrophotometer HITACHI U-2000 (Hitachi), and L-glutamic acid concentration was measured by using a glutamic acid analyzer AS-210 (Asahi Chemical Industry).

Table 18

Composition of medium for seed culture	
Medium composition	Concentration
Glucose	30 g/l
Ammonium sulfate	15 g/l
KH ₂ PO ₄	1 g/l
MgSO ₄ ·7H ₂ O	0.4 g/l
FeSO ₄ ·7H ₂ O	0.01 g/l
MnSO ₄ ·7H ₂ O	0.01 g/l
Soybean protein hydrolysate	0.48 g/l
Thiamin hydrochloride	200 µg/l
Biotin	10 µg/l
AZ20R	0.02 ml/l
CaCO ₃ (separately sterilized)	1 g/L
pH 8.0 (adjusted with KOH)	

Table 19

Composition of medium for main culture	
Medium composition	Concentration
Glucose	60 g/l
Ammonium sulfate	30 g/l
KH ₂ PO ₄	1 g/l
MgSO ₄ ·7H ₂ O	0.4 g/l
FeSO ₄ ·7H ₂ O	0.01 g/l
MnSO ₄ ·7H ₂ O	0.01 g/l
Soybean protein hydrolysate	0.48 g/l
Thiamin hydrochloride	200 µg/l
AZ20R	0.02 ml/l
CaCO ₃ (separately sterilized)	1 g/L
PH 8.0 (adjusted with KOH)	

[0180] The results of the culture are shown in Table 20 and Table 21. At 37°C, the *gdh*-amplified strain showed higher saccharide consuming rate, better growth and higher attained OD compared with the parent strain, the AJ12310 strain. Moreover, both of the L-glutamic acid accumulation and the yield were markedly improved, i.e., 5-7%, at 37°C. Also at 44°C, the yield was improved, and the attained OD increased. On the other hand, it was confirmed that accumulation of α -ketoglutaric acid was decreased in the *gdh*-amplified strain. These results demonstrate that the amplification of *gdh* is effective for improvement in L-glutamic acid yield and reduction of byproduct.

Table 20

Culture result of <i>gdh</i> -amplified strain (37°C)				
	OD ₆₂₀ (51x)	L-Glu accumulation (g/dl)	L-Glu yield (%)	α -KG (mg/dl)
AJ12310	0.58	1.74	30.7	53.9
AJ12310/PYGDH	0.65	2.23	39.3	4.1

Table 21

Culture result of <i>gdh</i> -amplified strain (44°C)			
	OD ₆₂₀ (51x)	L-Glu accumulation (g/dl)	L-Glu yield (%)
AJ12310	0.63	1.70	26.7
AJ12310/pYGDH	0.71	1.79	27.8

Example 9: L-glutamic acid production by *gltA* gene-amplified strain

<1> Construction of plasmid carrying *gltA* gene derived from *Corynebacterium thermoaminogenes*

[0181] Based on the *gltA* gene sequence of the AJ12310 strain shown in SEQ ID NO: 89, the primers shown in SEQ ID NO: 107 and SEQ ID NO: 108 were synthesized.

[0182] Separately, chromosomal DNA of the AJ12310 strain was prepared by using a Bacterial Genome DNA Purification Kit (Advanced Genetic Technologies Corp.). Sterilized water was added to 0.5 μ g of this chromosomal DNA, 10 pmol each of the aforementioned oligonucleotides, 8 μ l of dNTP mixture (2.5 mM each), 10 μ l of 10 x Pyrobest-Taq Buffer (Takara Shuzo) and 2 U of Pyrobest Taq (Takara Shuzo) to prepare a PCR reaction mixture in a total volume of 100 μ l. PCR was performed with a cycle of denaturation at 94°C for 30 seconds, association at 45°C for 30 seconds and extension reaction at 72°C for 3 minutes, which was repeated for 30 cycles, by using the above reaction mixture and a thermal cycler TP240 (Takara Shuzo) to amplify a DNA fragment of about 2 kb containing the *gltA* gene and its promoter. The obtained amplified fragment was digested with *KpnI* (Takara Shuzo), mixed with pHSG299 (Takara Shuzo) fully digested with *KpnI* and ligated to it. A DNA Ligation Kit Ver.2 produced by Takara Shuzo was used for the ligation reaction. After the ligation, competent cells of *Escherichia coli* JM109 (produced by Takara Shuzo) were transformed with the ligation product, plated on L medium (10 g/l of Bacto-trypton, 5 g/l of Bacto-yeast extract, 5 g/l of NaCl, 15 g/l of agar, pH 7.2) containing 10 μ g/ml of IPTG (isopropyl- β -D-thiogalactopyranoside), 40 μ g/ml of X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactoside) and 40 μ g/ml of chloramphenicol, and cultured overnight. The emerged white colonies were picked up and subjected to single colony separation to obtain transformants.

[0183] Plasmids were prepared from the transformants by the alkali method (Text for Bioengineering Experiments, Edited by the Society for Bioscience and Bioengineering, Japan, p.105, Baifukan, 1992) and their restriction maps were prepared. A plasmid having a restriction map equivalent to that shown in Fig. 22 was designated as pHSG299YCS.

[0184] A replication origin that is replicable in coryneform bacteria was introduced into this pHSG299YCS. Specifically, pXC4 was digested with a restriction enzyme *XbaI* to obtain a fragment containing a replication origin derived from pHM1519, and it was mixed with pHSG299YCS fully digested with *XbaI* and ligated to it. Plasmids were prepared in the same manner as above and a plasmid having a restriction map equivalent to that shown in Fig. 22 was designated as pYCS.

<2> Transfer of plasmid carrying *gltA* gene into AJ12310 strain

[0185] The plasmid produced above was introduced into the *Corynebacterium thermoaminogenes* AJ12310 strain

to prepare a *gltA* gene-amplified strain. The transformation was performed in the same manner as Example 5, and a transformant was selected on CM-2B agar medium containing 25 µg/ml kanamycin to obtain AJ12310/pYCS.

<3> L-glutamic acid production by *gltA*-amplified strain

[0186] The AJ12310 strain and the *gltA* gene-amplified strain obtained above, AJ12310/pYCS strain, both of which were grown on CM-2B agar medium, were cultured in the same manner as in Example 8. The results of the culture are shown in Table 22 and Table 23. Both at the culture temperatures, 37°C and 44°C, the CS-enhanced strain showed improved glutamic acid accumulation compared with the parent strain. Further, the *gltA*-amplified strain showed decreased L-aspartic acid and L-lysine, which are synthesized from oxaloacetic acid.

[0187] These results demonstrate that the amplification of *gltA* is effective for improvement of L-glutamic acid yield and reduction of byproduct.

Table 22

Culture result of <i>gltA</i> -amplified strain (37°C)				
	L-Glu accumulation (g/dl)	Yield (%)	L-Asp accumulation (mg/dl)	L-Lys accumulation (mg/dl)
AJ12310	1.79	31.9	11.8	11.0
AJ12310/pYCS	2.04	36.5	8.1	7.3

Table 23

Culture result of <i>gltA</i> -amplified strain (44°C)					
	OD	L-Glu accumulation (g/dl)	Yield (%)	L-Asp accumulation (mg/dl)	L-Lys Accumulation (mg/dl)
AJ12310	0.58	1.38	21.8	23.3	29.2
AJ12310/pYCS	0.65	1.84	28.8	14.1	17.2

[Explanation of Sequence Listing]

[0188]

SEQ ID NO: 1: *aceA*, nucleotide sequence
 SEQ ID NO: 2: *aceA*, amino acid sequence
 SEQ ID NO: 3: *accBC*, nucleotide sequence
 SEQ ID NO: 4: *accBC*, amino acid sequence
 SEQ ID NO: 5: *dtSR1*, nucleotide sequence
 SEQ ID NO: 6: *dtSR1*, amino acid sequence
 SEQ ID NO: 7: *dtSR2*, nucleotide sequence
 SEQ ID NO: 8: *dtSR2*, amino acid sequence
 SEQ ID NO: 9: *pfk*, nucleotide sequence
 SEQ ID NO: 10: *pfk*, amino acid sequence
 SEQ ID NO: 11: *scrB* (AJ12340), nucleotide sequence
 SEQ ID NO: 12: *scrB* (AJ12340), amino acid sequence
 SEQ ID NO: 13: *scrB* (AJ12309), nucleotide sequence
 SEQ ID NO: 14: *scrB* (AJ12309), amino acid sequence
 SEQ ID NO: 15: *scrB* (AJ12310), nucleotide sequence
 SEQ ID NO: 16: *gluABCD*, nucleotide sequence
 SEQ ID NO: 17: *gluABCD*, amino acid sequence
 SEQ ID NO: 18: *gluABCD*, amino acid sequence
 SEQ ID NO: 19: *gluABCD*, amino acid sequence
 SEQ ID NO: 20: *gluABCD*, amino acid sequence
 SEQ ID NO: 21: *pdhA*, nucleotide sequence
 SEQ ID NO: 22: *pdhA*, amino acid sequence

SEQ ID NO: 23: *pc*, nucleotide sequence
 SEQ ID NO: 24: *pc*, amino acid sequence
 SEQ ID NO: 25: *ppc*, nucleotide sequence
 SEQ ID NO: 26: *ppc*, amino acid sequence
 SEQ ID NO: 27: *acn*, nucleotide sequence
 SEQ ID NO: 28: *acn*, amino acid sequence
 SEQ ID NO: 29: *icd*, nucleotide sequence
 SEQ ID NO: 30: *icd*, amino acid sequence
 SEQ ID NO: 31: *lpd*, nucleotide sequence
 SEQ ID NO: 32: *lpd*, amino acid sequence
 SEQ ID NO: 33: *odhA*, nucleotide sequence
 SEQ ID NO: 34: *odhA*, amino acid sequence
 SEQ ID NO: 79: *gdh* (AJ12310), nucleotide sequence
 SEQ ID NO: 80: *gdh* (AJ12310), amino acid sequence
 SEQ ID NO: 81: *gdh* (2256), nucleotide sequence
 SEQ ID NO: 82: *gdh* (2256), amino acid sequence
 SEQ ID NO: 89: *gltA* (AJ12310), nucleotide sequence
 SEQ ID NO: 90: *gltA* (AJ12310), amino acid sequence
 SEQ ID NO: 91: *gltA* (2256), nucleotide sequence
 SEQ ID NO: 92: *gltA* (2256), amino acid sequence
 SEQ ID NO: 93: *scrB* (AJ12309), nucleotide sequence
 SEQ ID NO: 94: *scrB* (AJ12309), amino acid sequence

Industrial Applicability

[0189] According to the present invention, genes coding for enzymes of amino acid biosynthetic pathway derived from *Corynebacterium thermoaminogenes*, or genes coding for proteins involved in the amino acid uptake into cells.

[0190] The genes of the present invention can be utilized for the production of the aforementioned enzymes or proteins, or the breeding of amino acid producing bacteria.

SEQUENCE LISTING

<110> Ajinomoto Co., Inc.

<120> Genes for Heat resistant Enzymes of Amino Acid
Biosynthetic Pathway Derived from Thermophilic
Coryneform Bacteria

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 50 gggacgata cccccgggta cggclacat tccaaaac atg acc att lcc tca cct 356

Met Thr Ile Ser Ser Pro
 1 5

11g att gac gic gct aac ctg cca gac atc aac acc acc gcc ggc aag 404
 55 Leu Ile Asp Val Ala Asn Leu Pro Asp Ile Asn Thr Thr Ala Gly Lys

	10	15	20	
	atc gcc gac ctg aag gcc cgc cgg ggc gaa gcc cac ttc ccc atg ggt	452		
5	Ile Ala Asp Leu Lys Ala Arg Arg Ala Glu Ala His Phe Pro Met Gly			
	25 30 35			
	gaa aag gcc gta gag aag gtc cac ggc gcc aac cgc ctc acc ggc cgc	500		
	Glu Lys Ala Val Glu Lys Val His Ala Ala Asn Arg Leu Thr Ala Arg			
10	40 45 50			
	gaa cga ctt gac tac ctg ctc gat gaa ggc tcc ttc atc gaa acc gat	548		
	Glu Arg Leu Asp Tyr Leu Leu Asp Glu Gly Ser Phe Ile Glu Thr Asp			
	55 60 65 70			
15	cag ctc gca cgc cac cgc acc acc ggc ttc ggc ctg ggc aac aag cga	596		
	Gln Leu Ala Arg His Arg Thr Thr Ala Phe Gly Leu Gly Asn Lys Arg			
	75 80 85			
	ccg gcc acc gac ggc atc gtc acc ggc tgg ggc acc atc gac ggc cgc	644		
20	Pro Ala Thr Asp Gly Ile Val Thr Gly Trp Gly Thr Ile Asp Gly Arg			
	90 95 100			
	gag gtc tgc atc ttc tcc cag gac ggc acc gtc ttc ggt ggc gca ctc	692		
	Glu Val Cys Ile Phe Ser Gln Asp Gly Thr Val Phe Gly Gly Ala Leu			
25	105 110 115			
	ggt gag gtc tac ggc gag aag atg atc aag atc atg gag ctg gcc atc	740		
	Gly Glu Val Tyr Gly Glu Lys Met Ile Lys Ile Met Glu Leu Ala Ile			
	120 125 130			
30	gac acc ggc cgc cca ctc atc ggc ctg tac gag ggt gca ggt gcc cgc	788		
	Asp Thr Gly Arg Pro Leu Ile Gly Leu Tyr Glu Gly Ala Gly Ala Arg			
	135 140 145 150			
	atc cag gac ggt ggc gtc tcc ctc gac ttc atc tcc cag acc ttc tat	836		
35	Ile Gln Asp Gly Ala Val Ser Leu Asp Phe Ile Ser Gln Thr Phe Tyr			
	155 160 165			
	cag aac atc cag gcc tcc ggc gtg atc ccg cag atc tcc gtg atc atg	884		
	Gln Asn Ile Gln Ala Ser Gly Val Ile Pro Gln Ile Ser Val Ile Met			
40	170 175 180			
	ggt gcc tgc gcc ggt ggc aac gcc tac ggc ccg gcc ctg acc gac ttc	932		
	Gly Ala Cys Ala Gly Gly Asn Ala Tyr Gly Pro Ala Leu Thr Asp Phe			
	185 190 195			
45	gtg gtc atg gtg gac aag acc tgc aag atg ttc gtc acc ggc ccc gat	980		
	Val Val Met Val Asp Lys Thr Ser Lys Met Phe Val Thr Gly Pro Asp			
	200 205 210			
	gtg atc aag acc gtc acc ggc gag gag atc acc cag gag gag ctc ggc	1028		
50	Val Ile Lys Thr Val Thr Gly Glu Glu Ile Thr Gln Glu Glu Leu Gly			
	215 220 225 230			
	gga gca acc acc cac atg gtc acc gcc ggc aac tcc cac tac acc gtc	1076		
	Gly Ala Thr Thr His Met Val Thr Ala Gly Asn Ser His Tyr Thr Val			
55	235 240 245			

gcc acc gat gag gag gcc ctc gac tgg gtc cag gac ctc atc tcc ttc 1124
 Ala Thr Asp Glu Glu Ala Leu Asp Trp Val Gln Asp Leu Ile Ser Phe
 250 255 260
 5 ctc ccc tcc aac aat cgc tcc tac gcc ccg gtg gag gag ttc gac gag 1172
 Leu Pro Ser Asn Asn Arg Ser Tyr Ala Pro Val Glu Glu Phe Asp Glu
 265 270 275
 10 gag gac ggt ggc atc gcc gag aac atc acc gcc gat gac ctg aag ctg 1220
 Glu Asp Gly Gly Ile Ala Glu Asn Ile Thr Ala Asp Asp Leu Lys Leu
 280 285 290
 gat gag atc atc ccg gat tcc gcc acc gtg ccc tat gat gtc cgc gac 1268
 15 Asp Glu Ile Ile Pro Asp Ser Ala Thr Val Pro Tyr Asp Val Arg Asp
 295 300 305 310
 gtc atc cag tgc ctg acc gac gac ggt gag tac ctg gag atc cag gcc 1316
 Val Ile Gln Cys Leu Thr Asp Asp Gly Glu Tyr Leu Glu Ile Gln Ala
 315 320 325
 20 gac cga gcc gag aat gtc gtc atc gcc ttc ggc cgc atc gag ggc cag 1364
 Asp Arg Ala Glu Asn Val Val Ile Ala Phe Gly Arg Ile Glu Gly Gln
 330 335 340
 25 tcc gtc ggt ttc gtc gcc aac cag ccg acc cag ttc gcc ggc tgc ctg 1412
 Ser Val Gly Phe Val Ala Asn Gln Pro Thr Gln Phe Ala Gly Cys Leu
 345 350 355
 gac atc gac tcc tcc gag aag gca gcc cgc ttc gtc cgc acc tgc gat 1460
 30 Asp Ile Asp Ser Ser Glu Lys Ala Ala Arg Phe Val Arg Thr Cys Asp
 360 365 370
 gcc ttc aac atc ccg atc gtc atg ctt gtc gac gtc ccc ggc ttc ctc 1508
 Ala Phe Asn Ile Pro Ile Val Met Leu Val Asp Val Pro Gly Phe Leu
 35 375 380 385 390
 ccc ggt gcc ggc cag gag tac ggc ggc atc ctg cgt cgt ggc gcc aaa 1556
 Pro Gly Ala Gly Gln Glu Tyr Gly Gly Ile Leu Arg Arg Gly Ala Lys
 395 400 405
 40 ctg ctc tac gcc tac ggt gag gcc acc gtc ccg aag atc acc gtg acc 1604
 Leu Leu Tyr Ala Tyr Gly Glu Ala Thr Val Pro Lys Ile Thr Val Thr
 410 415 420
 45 atg cgc aag gcc tac ggc ggt gcg tac tgt gtc atg gga tcc aag ggt 1652
 Met Arg Lys Ala Tyr Gly Gly Ala Tyr Cys Val Met Gly Ser Lys Gly
 425 430 435
 ctg ggc gca gac atc aac ctg gcc tgg ccg acc gcg cag atc gcc gtc 1700
 50 Leu Gly Ala Asp Ile Asn Leu Ala Trp Pro Thr Ala Gln Ile Ala Val
 440 445 450
 atg ggt gcc gcc ggc gcg gtc cag ttc atc tac cgc aag gag ctc atg 1748
 Met Gly Ala Ala Gly Ala Val Gln Phe Ile Tyr Arg Lys Glu Leu Met
 455 460 465 470
 55 gcc gcl gat gcc aag ggc ctg gac acc gtc gcc ctg gcc cag tcc ttc 1796

Ala Ala Asp Ala Lys Gly Leu Asp Thr Val Ala Leu Ala Gln Ser Phe

475

480

485

gag cgt gag tac gag gac cac atg ctc aac ccg tac ctg gcg gcc gag 1844

Glu Arg Glu Tyr Glu Asp His Met Leu Asn Pro Tyr Leu Ala Ala Glu

490

495

500

cgt ggc ctc atc gac gcg gtg atc ctg ccg tcc gag acc cgt ggc cag 1892

Arg Gly Leu Ile Asp Ala Val Ile Leu Pro Ser Glu Thr Arg Gly Gln

505

510

515

atc gca cgc aac ctg cgt ctg ctc aag cac aag aat gtc tcc cgc cct 1940

Ile Ala Arg Asn Leu Arg Leu Leu Lys His Lys Asn Val Ser Arg Pro

520

525

530

gcc cgc aag cac ggc aac atg cca ctg taagcaccg ggaccacccc 1987

Ala Arg Lys His Gly Asn Met Pro Leu

535 540

ctacgcccgc acccaggcc ctltgctggc agglgcgggc gctgtgcgtt ttccgcgcct 2047

gccgacgccc ggccccctgc cctgtgatgc gatctgcgga tgtgatctgc gccgcgcga 2107

actccccctgg ttgaacccctg c 2128

<210> 6

<211> 543

<212> PRT

<213> Corynebacterium thermoaminogenes

<400> 6

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1

5

10

15

Asn Thr Thr Ala Gly Lys Ile Ala Asp Leu Lys Ala Arg Arg Ala Glu

20

25

30

Ala His Phe Pro Met Gly Glu Lys Ala Val Glu Lys Val His Ala Ala

35

40

45

Asn Arg Leu Thr Ala Arg Glu Arg Leu Asp Tyr Leu Leu Asp Glu Gly

50

55

60

Ser Phe Ile Glu Thr Asp Gln Leu Ala Arg His Arg Thr Thr Ala Phe

65

70

75

80

Gly Leu Gly Asn Lys Arg Pro Ala Thr Asp Gly Ile Val Thr Gly Trp

85

90

95

Gly Thr Ile Asp Gly Arg Glu Val Cys Ile Phe Ser Gln Asp Gly Thr

100

105

110

Val Phe Gly Gly Ala Leu Gly Glu Val Tyr Gly Glu Lys Met Ile Lys

115

120

125

Ile Met Glu Leu Ala Ile Asp Thr Gly Arg Pro Leu Ile Gly Leu Tyr

130

135

140

Glu Gly Ala Gly Ala Arg Ile Gln Asp Gly Ala Val Ser Leu Asp Phe

145 150 155 160
 Ile Ser Gln Thr Phe Tyr Gln Asn Ile Gln Ala Ser Gly Val Ile Pro
 5 165 170 175
 Gln Ile Ser Val Ile Met Gly Ala Cys Ala Gly Gly Asn Ala Tyr Gly
 180 185 190
 Pro Ala Leu Thr Asp Phe Val Val Met Val Asp Lys Thr Ser Lys Met
 10 195 200 205
 Phe Val Thr Gly Pro Asp Val Ile Lys Thr Val Thr Gly Glu Glu Ile
 210 215 220
 Thr Gln Glu Glu Leu Gly Gly Ala Thr Thr His Met Val Thr Ala Gly
 15 225 230 235 240
 Asn Ser His Tyr Thr Val Ala Thr Asp Glu Glu Ala Leu Asp Trp Val
 245 250 255
 Gln Asp Leu Ile Ser Phe Leu Pro Ser Asn Asn Arg Ser Tyr Ala Pro
 20 260 265 270
 Val Glu Glu Phe Asp Glu Glu Asp Gly Gly Ile Ala Glu Asn Ile Thr
 275 280 285
 Ala Asp Asp Leu Lys Leu Asp Glu Ile Ile Pro Asp Ser Ala Thr Val
 25 290 295 300
 Pro Tyr Asp Val Arg Asp Val Ile Gln Cys Leu Thr Asp Asp Gly Glu
 305 310 315 320
 Tyr Leu Glu Ile Gln Ala Asp Arg Ala Glu Asn Val Val Ile Ala Phe
 30 325 330 335
 Gly Arg Ile Glu Gly Gln Ser Val Gly Phe Val Ala Asn Gln Pro Thr
 340 345 350
 Gln Phe Ala Gly Cys Leu Asp Ile Asp Ser Ser Glu Lys Ala Ala Arg
 35 355 360 365
 Phe Val Arg Thr Cys Asp Ala Phe Asn Ile Pro Ile Val Met Leu Val
 370 375 380
 Asp Val Pro Gly Phe Leu Pro Gly Ala Gly Gln Glu Tyr Gly Gly Ile
 40 385 390 395 400
 Leu Arg Arg Gly Ala Lys Leu Leu Tyr Ala Tyr Gly Glu Ala Thr Val
 405 410 415
 Pro Lys Ile Thr Val Thr Met Arg Lys Ala Tyr Gly Gly Ala Tyr Cys
 45 420 425 430
 Val Met Gly Ser Lys Gly Leu Gly Ala Asp Ile Asn Leu Ala Trp Pro
 435 440 445
 Thr Ala Gln Ile Ala Val Met Gly Ala Ala Gly Ala Val Gln Phe Ile
 50 450 455 460
 Tyr Arg Lys Glu Leu Met Ala Ala Asp Ala Lys Gly Leu Asp Thr Val
 465 470 475 480
 Ala Leu Ala Gln Ser Phe Glu Arg Glu Tyr Glu Asp His Met Leu Asn
 55 485 490 495

Pro Tyr Leu Ala Ala Glu Arg Gly Leu Ile Asp Ala Val Ile Leu Pro

500

505

510

Ser Glu Thr Arg Gly Gln Ile Ala Arg Asn Leu Arg Leu Leu Lys His

515

520

525

Lys Asn Val Ser Arg Pro Ala Arg Lys His Gly Asn Met Pro Leu

530

535

540

<210> 7

<211> 2076

<212> DNA

<213> *Corynebacterium thermoaminogenes*

<220>

<221> CDS

<222> (412)..(2022)

<400> 7

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acggggggga ggagglicaca taggccatac gctgcacitl lgaigaagtg tggcgagatc 180

gaccgggcaa atcigggaaa taaggggcci ggigaactag catlccccit agcgaagggt 240

gagcaticcg gaccccgga tgcaccaacc ggicgtaaat lcalgtgccg ccacagtcac 300

ctcaccaggg gatcggaacc agcccagcct galiccgcg tgcaggacct caccgigaac 360

aagtccccg c attactcaca gaactcacac caggatitag actaagaac c atg act 417

Met Thr

1

gca gca acg aca gca cct gat cag acc acc acc gcc ggc aaa ctc ggc 465

Ala Ala Thr Thr Ala Pro Asp Leu Thr Thr Thr Ala Gly Lys Leu Ala

5

10

15

gat ctc cgc gcc cgc ctt tcc gag acc cag gcc ccc atg ggt cag gcc 513

Asp Leu Arg Ala Arg Leu Ser Glu Thr Gln Ala Pro Met Gly Gln Ala

20

25

30

tcg gtc gag aag gtc cac gag gca ggg aag aag acc gca cgc gag cgc 561

Ser Val Glu Lys Val His Glu Ala Gly Lys Lys Thr Ala Arg Glu Arg

35

40

45

50

alc gag tac ctc ctc gat gag ggc tcc ttc gtt gag gtc gat gcc ctc 609

Ile Glu Tyr Leu Leu Asp Glu Gly Ser Phe Val Glu Val Asp Ala Leu

55

60

65

gcc cgc cac cgt tcc aag aac ttc ggc ctc gac tcc aag cgc ccg gtc 657

Ala Arg His Arg Ser Lys Asn Phe Gly Leu Asp Ser Lys Arg Pro Val

70

75

80

acc gac ggt gtc gtc acc ggt tac ggc acc alc gac gga cgc aag gtc 705

Thr Asp Gly Val Val Thr Gly Tyr Gly Thr Ile Asp Gly Arg Lys Val

	85	90	95	
	tgc gtc ttc tcc cag gac ggc gct atc ttc ggc ggt gcc ctc ggt gag			753
5	Cys Val Phe Ser Gln Asp Gly Ala Ile Phe Gly Gly Ala Leu Gly Glu			
	100	105	110	
	gtc tac ggc gag aag atc gtc aag atc atg gac ctg gcc atc aag acc			801
10	Val Tyr Gly Glu Lys Ile Val Lys Ile Met Asp Leu Ala Ile Lys Thr			
	115	120	125	130
	ggt gtc ccc ctc atc ggc atc aac gag ggc gcc ggc gcc cgc atc cag			849
	Gly Val Pro Leu Ile Gly Ile Asn Glu Gly Ala Gly Ala Arg Ile Gln			
	135	140	145	
15	gaa ggc gtt gtc tcc ctg ggc ctg tac tcc cag atc ttc tac cgc aac			897
	Glu Gly Val Val Ser Leu Gly Leu Tyr Ser Gln Ile Phe Tyr Arg Asn			
	150	155	160	
	acc cag gca tcc ggt gtc atc cca cag atc tcc ctc atc atg ggt gcc			945
20	Thr Gln Ala Ser Gly Val Ile Pro Gln Ile Ser Leu Ile Met Gly Ala			
	165	170	175	
	tgc gcc ggt ggc cat gtg tac tcc ccc gcc ctg acc gac ttc atc atc			993
	Cys Ala Gly Gly His Val Tyr Ser Pro Ala Leu Thr Asp Phe Ile Ile			
25	180	185	190	
	atg gtg gac aag acc tcc aag atg ttc atc acc ggc ccc gac gtg atc			1041
	Met Val Asp Lys Thr Ser Lys Met Phe Ile Thr Gly Pro Asp Val Ile			
	195	200	205	210
30	aag acc gtc acc ggc gag gag gtc acc cag gag gaa ctg ggt ggt gcc			1089
	Lys Thr Val Thr Gly Glu Glu Val Thr Gln Glu Glu Leu Gly Gly Ala			
	215	220	225	
	tac acc cac atg gcc cag tcc ggc acc tgc cac tac acc gca gcc gat			1137
35	Tyr Thr His Met Ala Gln Ser Gly Thr Ser His Tyr Thr Ala Ala Asp			
	230	235	240	
	gac tcc gat gcc ctc gac tgg gtc cgt gag ctg gtc agc tac ctg ccg			1185
	Asp Ser Asp Ala Leu Asp Trp Val Arg Glu Leu Val Ser Tyr Leu Pro			
40	245	250	255	
	tcc aac aac cgt gcg gag acc cca cgc cag gac gcc gac atc atg gtg			1233
	Ser Asn Asn Arg Ala Glu Thr Pro Arg Gln Asp Ala Asp Ile Met Val			
	260	265	270	
45	ggc tcc atc aag gag aac atc acc gag acc gac ctc gaa ctc gac acc			1281
	Gly Ser Ile Lys Glu Asn Ile Thr Glu Thr Asp Leu Glu Leu Asp Thr			
	275	280	285	290
	ctg atc ccg gat tcc ccg aac cag ccg tac gac atg aag gac gtc atc			1329
50	Leu Ile Pro Asp Ser Pro Asn Gln Pro Tyr Asp Met Lys Asp Val Ile			
	295	300	305	
	acc cgc atc gtc gat gat gcc gag ttc ttc gag atc cag gag ggt tac			1377
55	Thr Arg Ile Val Asp Asp Ala Glu Phe Phe Glu Ile Gln Glu Gly Tyr			
	310	315	320	

	gcc gag aac atc atc tgc ggt ttc gcc cgc gtc gag ggt cgt gcc gtg	1425
	Ala Glu Asn Ile Ile Cys Gly Phe Ala Arg Val Glu Gly Arg Ala Val	
	325 330 335	
5	ggt atc gtc gcc aac cag ccg atg cag ttc gcc ggc tgc ctg gac atc	1473
	Gly Ile Val Ala Asn Gln Pro Met Gln Phe Ala Gly Cys Leu Asp Ile	
	340 345 350	
10	aag gca tcc gag aag gcc gcc cgc ttc atc cgc acc tgt gac gcc ttc	1521
	Lys Ala Ser Glu Lys Ala Ala Arg Phe Ile Arg Thr Cys Asp Ala Phe	
	355 360 365 370	
15	aac atc ccg atc atc gag ctt gtc gac gtc cca ggc ttc ctc ccg ggc	1569
	Asn Ile Pro Ile Ile Glu Leu Val Asp Val Pro Gly Phe Leu Pro Gly	
	375 380 385	
	acc aac cag gag ttc gac ggc atc atc cgt cgc ggc gcg aag ctg ctc	1617
	Thr Asn Gln Glu Phe Asp Gly Ile Ile Arg Arg Gly Ala Lys Leu Leu	
	390 395 400	
20	tac gcc tac gcc gag gcc acc gtc ggc aag atc acc gtg atc acc cgc	1665
	Tyr Ala Tyr Ala Glu Ala Thr Val Gly Lys Ile Thr Val Ile Thr Arg	
	405 410 415	
25	aag tcc tac ggc ggt gcc tac tgc gtg atg ggc tcc aag gac atg ggt	1713
	Lys Ser Tyr Gly Gly Ala Tyr Cys Val Met Gly Ser Lys Asp Met Gly	
	420 425 430	
30	gcg gac ctc gtc ttc gca tgg ccc acc gcg cag atc gcc gtc atg ggt	1761
	Ala Asp Leu Val Phe Ala Trp Pro Thr Ala Gln Ile Ala Val Met Gly	
	435 440 445 450	
	gcc tcc ggt gcc gtc ggc ttc atc tac cgc aag gag ctc aag cag gct	1809
	Ala Ser Gly Ala Val Gly Phe Ile Tyr Arg Lys Glu Leu Lys Gln Ala	
	455 460 465	
35	gca gcg gcc ggc gag gat gtc acc gcg ctg atg aag aag tac gag cag	1857
	Ala Ala Ala Gly Glu Asp Val Thr Ala Leu Met Lys Lys Tyr Glu Gln	
	470 475 480	
40	gag tac gag gag acc ctg gtc aac ccg tac atg gct gca gag cgt ggc	1905
	Glu Tyr Glu Glu Thr Leu Val Asn Pro Tyr Met Ala Ala Glu Arg Gly	
	485 490 495	
45	tac gtc gac gcc gtc atc cca cca tcc gag acc cgt ggt cag atc atc	1953
	Tyr Val Asp Ala Val Ile Pro Pro Ser Glu Thr Arg Gly Gln Ile Ile	
	500 505 510	
	gag ggt ctg cgt ctg ctc gac cgc aag gtg gtc aac gtc ccg gcc aag	2001
	Glu Gly Leu Arg Leu Leu Asp Arg Lys Val Val Asn Val Pro Ala Lys	
	515 520 525 530	
50	aag cac ggt aac atc ccg ctg taaaccgtct tccctccgg caccacgccg	2052
	Lys His Gly Asn Ile Pro Leu	
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<210> 8

<211> 537

<212> PRT

<213> *Corynebacterium thermoaminogenes*

<400> 8

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 20 25 30
 Gln Ala Ser Val Glu Lys Val His Glu Ala Gly Lys Lys Thr Ala Arg
 35 40 45
 Glu Arg Ile Glu Tyr Leu Leu Asp Glu Gly Ser Phe Val Glu Val Asp
 50 55 60
 Ala Leu Ala Arg His Arg Ser Lys Asn Phe Gly Leu Asp Ser Lys Arg
 65 70 75 80
 Pro Val Thr Asp Gly Val Val Thr Gly Tyr Gly Thr Ile Asp Gly Arg
 85 90 95
 Lys Val Cys Val Phe Ser Gln Asp Gly Ala Ile Phe Gly Gly Ala Leu
 100 105 110
 Gly Glu Val Tyr Gly Glu Lys Ile Val Lys Ile Met Asp Leu Ala Ile
 115 120 125
 Lys Thr Gly Val Pro Leu Ile Gly Ile Asn Glu Gly Ala Gly Ala Arg
 130 135 140
 Ile Gln Glu Gly Val Val Ser Leu Gly Leu Tyr Ser Gln Ile Phe Tyr
 145 150 155 160
 Arg Asn Thr Gln Ala Ser Gly Val Ile Pro Gln Ile Ser Leu Ile Met
 165 170 175
 Gly Ala Cys Ala Gly Gly His Val Tyr Ser Pro Ala Leu Thr Asp Phe
 180 185 190
 Ile Ile Met Val Asp Lys Thr Ser Lys Met Phe Ile Thr Gly Pro Asp
 195 200 205
 Val Ile Lys Thr Val Thr Gly Glu Glu Val Thr Gln Glu Glu Leu Gly
 210 215 220
 Gly Ala Tyr Thr His Met Ala Gln Ser Gly Thr Ser His Tyr Thr Ala
 225 230 235 240
 Ala Asp Asp Ser Asp Ala Leu Asp Trp Val Arg Glu Leu Val Ser Tyr
 245 250 255
 Leu Pro Ser Asn Asn Arg Ala Glu Thr Pro Arg Gln Asp Ala Asp Ile
 260 265 270
 Met Val Gly Ser Ile Lys Glu Asn Ile Thr Glu Thr Asp Leu Glu Leu
 275 280 285

	Asp	Thr	Leu	Ile	Pro	Asp	Ser	Pro	Asn	Gln	Pro	Tyr	Asp	Met	Lys	Asp
	290						295					300				
5	Val	Ile	Thr	Arg	Ile	Val	Asp	Asp	Ala	Glu	Phe	Phe	Glu	Ile	Gln	Glu
	305					310					315					320
	Gly	Tyr	Ala	Glu	Asn	Ile	Ile	Cys	Gly	Phe	Ala	Arg	Val	Glu	Gly	Arg
					325					330					335	
10	Ala	Val	Gly	Ile	Val	Ala	Asn	Gln	Pro	Met	Gln	Phe	Ala	Gly	Cys	Leu
				340					345					350		
	Asp	Ile	Lys	Ala	Ser	Glu	Lys	Ala	Ala	Arg	Phe	Ile	Arg	Thr	Cys	Asp
			355					360					365			
15	Ala	Phe	Asn	Ile	Pro	Ile	Ile	Glu	Leu	Val	Asp	Val	Pro	Gly	Phe	Leu
	370						375					380				
	Pro	Gly	Thr	Asn	Gln	Glu	Phe	Asp	Gly	Ile	Ile	Arg	Arg	Gly	Ala	Lys
	385					390					395					400
20	Leu	Leu	Tyr	Ala	Tyr	Ala	Glu	Ala	Thr	Val	Gly	Lys	Ile	Thr	Val	Ile
				405					410						415	
	Thr	Arg	Lys	Ser	Tyr	Gly	Gly	Ala	Tyr	Cys	Val	Met	Gly	Ser	Lys	Asp
				420				425						430		
25	Met	Gly	Ala	Asp	Leu	Val	Phe	Ala	Trp	Pro	Thr	Ala	Gln	Ile	Ala	Val
		435					440						445			
	Met	Gly	Ala	Ser	Gly	Ala	Val	Gly	Phe	Ile	Tyr	Arg	Lys	Glu	Leu	Lys
		450				455						460				
30	Gln	Ala	Ala	Ala	Ala	Gly	Glu	Asp	Val	Thr	Ala	Leu	Met	Lys	Lys	Tyr
	465					470					475					480
	Glu	Gln	Glu	Tyr	Glu	Glu	Thr	Leu	Val	Asn	Pro	Tyr	Met	Ala	Ala	Glu
				485					490						495	
35	Arg	Gly	Tyr	Val	Asp	Ala	Val	Ile	Pro	Pro	Ser	Glu	Thr	Arg	Gly	Gln
				500					505					510		
	Ile	Ile	Glu	Gly	Leu	Arg	Leu	Leu	Asp	Arg	Lys	Val	Val	Asn	Val	Pro
			515					520					525			
40	Ala	Lys	Lys	His	Gly	Asn	Ile	Pro	Leu							
		530					535									

<210> 9

<211> 1643

<212> DNA

<213> Corynebacterium thermoaminogenes

<220>

<221> CDS

<222> (326).. (1363)

<400> 9

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 5 clllgaglit calatccalg tcagacaglc laaccacict ciccagcgcg tccgaacatg 180
 ciggggcggc ggacaccaig tccgttcggg cgtlgccccg acgggggaaa atgcagcga 240
 gatgtgiccg atgtgggata aacccaccgg ttcgggcgig tcttcgggat caatggcaca 300
 gcattaacgg tglggggggg ttaat alg gga gcc alg cga att gcc act ctc 352
 10 Met Gly Ala Met Arg Ile Ala Thr Leu
 1 5
 acg tca ggc ggc gac igc ccc gga ctc aat gct gtc atc agg gga atc 400
 Thr Ser Gly Gly Asp Cys Pro Gly Leu Asn Ala Val Ile Arg Gly Ile
 15 10 15 20 25
 gtc cgt acc gca agt aat gaa ttc ggt tcc acc gtc gtc ggt tat cag 448
 Val Arg Thr Ala Ser Asn Glu Phe Gly Ser Thr Val Val Gly Tyr Gln
 30 35 40
 20 gac ggc tgg gag ggc ctc ctc gcg gac cga cgt gtt cag ctc tat gac 496
 Asp Gly Trp Glu Gly Leu Leu Ala Asp Arg Arg Val Gln Leu Tyr Asp
 45 50 55
 gat gag gac atc gac cgc atc ctg ctc cgc ggt gga aca atc ctg ggc 544
 25 Asp Glu Asp Ile Asp Arg Ile Leu Leu Arg Gly Gly Thr Ile Leu Gly
 60 65 70
 acc ggt cgt ctc cac ccc gac aag ttc aga gcc gga atc gac cag gtc 592
 Thr Gly Arg Leu His Pro Asp Lys Phe Arg Ala Gly Ile Asp Gln Val
 30 75 80 85
 aag gcg aat ctc gcc gat gcg gga att gac gca ctc atc ccg atc ggt 640
 Lys Ala Asn Leu Ala Asp Ala Gly Ile Asp Ala Leu Ile Pro Ile Gly
 90 95 100 105
 35 ggc gag ggc acc ctc aag gga gcg aag tgg ctc gcc gac aac ggc atc 688
 Gly Glu Gly Thr Leu Lys Gly Ala Lys Trp Leu Ala Asp Asn Gly Ile
 110 115 120
 ccc gtc gtc ggt gtc ccg aaa acc atc gac aat gat gtc aac ggc acg 736
 40 Pro Val Val Gly Val Pro Lys Thr Ile Asp Asn Asp Val Asn Gly Thr
 125 130 135
 gat ttc acc ttc ggt ttc gat tcc gcg gtc tct gtc gcc acc gac gcc 784
 Asp Phe Thr Phe Gly Phe Asp Ser Ala Val Ser Val Ala Thr Asp Ala
 45 140 145 150
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 Ile Asp Arg Leu His Thr Thr Ala Glu Ser His Asn Arg Val Met Ile
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 50 gtc gag gtc atg ggc cgc cac gtc ggt tgg atc gca ctg cat gcc ggc 880
 Val Glu Val Met Gly Arg His Val Gly Trp Ile Ala Leu His Ala Gly
 170 175 180 185
 atg gcc ggt gga gcc cac tac acc gtc atc ccc gag gtc ccc ttc gac 928
 55 Met Ala Gly Gly Ala His Tyr Thr Val Ile Pro Glu Val Pro Phe Asp

190 195 200
 atc tgc gag atc tgc aag cgt atg gaa cgt cgc ttc cag atg ggg gag 976
 Ile Ser Glu Ile Cys Lys Arg Met Glu Arg Arg Phe Gln Met Gly Glu
 205 210 215
 aag tac ggc atc atc gtc gtc gcg gag ggt gcc ctg ccc aag gag gga 1024
 Lys Tyr Gly Ile Ile Val Val Ala Glu Gly Ala Leu Pro Lys Glu Gly
 220 225 230
 acc atg gag ctg cgt gag ggg gag gtg gat cag ttc ggt cac aag acc 1072
 Thr Met Glu Leu Arg Glu Gly Glu Val Asp Gln Phe Gly His Lys Thr
 235 240 245
 ttc acc ggc atc ggc cag cag atc gcc gac gag gtg cac agg cgt ctg 1120
 Phe Thr Gly Ile Gly Gln Gln Ile Ala Asp Glu Val His Arg Arg Leu
 250 255 260 265
 ggt cat gat gtc cgc acc acg gtc ctg ggc cat atc cag cgt ggt ggc 1168
 Gly His Asp Val Arg Thr Thr Val Leu Gly His Ile Gln Arg Gly Gly
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 Thr Pro Thr Ala Phe Asp Arg Val Leu Ala Thr Arg Tyr Gly Val Arg
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 gcc gcg cgt gcc tgc cac gag ggt cag ttc aac acc gtg gtc gcg ctc 1264
 Ala Ala Arg Ala Cys His Glu Gly Gln Phe Asn Thr Val Val Ala Leu
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 aag ggg gag cgc atc cgg atg atc tcc ttc gat gag gcc gtg ggc acc 1312
 Lys Gly Glu Arg Ile Arg Met Ile Ser Phe Asp Glu Ala Val Gly Thr
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 ctg aag aag gtg ccg atg gaa cgc tgg gtg acc gcc cag gct atg ttc 1360
 Leu Lys Lys Val Pro Met Glu Arg Trp Val Thr Ala Gln Ala Met Phe
 330 335 340 345
 ggt tagtcaggcc gcatcccggt ttcgcgcgcc gcggggccgg gtttttcat 1413
 Gly
 gccccggaac acatcggtat gaaatcgiga taigcattac ttgacgggga agtgggggat 1473
 ccgtcaccic gcgttgicca actacagccc gcagcgccig cgggaaattct tcgagcaatc 1533
 cgccgattec cgggcccgic ccgtcgccgt ccaaccgcag tacaatctgc tggcccccg 1593
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 50 <213> Corynebacterium thermoaminogenes
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 5 Phe Gly Ser Thr Val Val Gly Tyr Gln Asp Gly Trp Glu Gly Leu Leu
 35 40 45
 Ala Asp Arg Arg Val Gln Leu Tyr Asp Asp Glu Asp Ile Asp Arg Ile
 50 55 60
 10 Leu Leu Arg Gly Gly Thr Ile Leu Gly Thr Gly Arg Leu His Pro Asp
 65 70 75 80
 Lys Phe Arg Ala Gly Ile Asp Gln Val Lys Ala Asn Leu Ala Asp Ala
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 15 Gly Ile Asp Ala Leu Ile Pro Ile Gly Gly Glu Gly Thr Leu Lys Gly
 100 105 110
 Ala Lys Trp Leu Ala Asp Asn Gly Ile Pro Val Val Gly Val Pro Lys
 115 120 125
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 130 135 140
 Ser Ala Val Ser Val Ala Thr Asp Ala Ile Asp Arg Leu His Thr Thr
 145 150 155 160
 25 Ala Glu Ser His Asn Arg Val Met Ile Val Glu Val Met Gly Arg His
 165 170 175
 Val Gly Trp Ile Ala Leu His Ala Gly Met Ala Gly Gly Ala His Tyr
 180 185 190
 30 Thr Val Ile Pro Glu Val Pro Phe Asp Ile Ser Glu Ile Cys Lys Arg
 195 200 205
 Met Glu Arg Arg Phe Gln Met Gly Glu Lys Tyr Gly Ile Ile Val Val
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 35 Ala Glu Gly Ala Leu Pro Lys Glu Gly Thr Met Glu Leu Arg Glu Gly
 225 230 235 240
 Glu Val Asp Gln Phe Gly His Lys Thr Phe Thr Gly Ile Gly Gln Gln
 245 250 255
 40 Ile Ala Asp Glu Val His Arg Arg Leu Gly His Asp Val Arg Thr Thr
 260 265 270
 Val Leu Gly His Ile Gln Arg Gly Gly Thr Pro Thr Ala Phe Asp Arg
 275 280 285
 45 Val Leu Ala Thr Arg Tyr Gly Val Arg Ala Ala Arg Ala Cys His Glu
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 Gly Gln Phe Asn Thr Val Val Ala Leu Lys Gly Glu Arg Ile Arg Met
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<213> Corynebacterium thermoaminogenes

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20  tgg gct cac acc acc acg ccg ttg acc gga ccg cag cga ttg cag tgg 96
    Trp Ala His Thr Thr Thr Pro Leu Thr Gly Pro Gln Arg Leu Gln Trp
    20      25      30
25  acg cac ctg ccc gat gct ctt tac ccg gat gta tcc tat gac ctg gat 144
    Thr His Leu Pro Asp Ala Leu Tyr Pro Asp Val Ser Tyr Asp Leu Asp
    35      40      45
30  gga tgc tat tcc ggc gga gcc gta ttt tct gac ggc acg ctt aaa ctt 192
    Gly Cys Tyr Ser Gly Gly Ala Val Phe Ser Asp Gly Thr Leu Lys Leu
    50      55      60
35  ttc tac acc ggc aac cga aaa att gac ggc aag cgc cgc gcc acc caa 240
    Phe Tyr Thr Gly Asn Arg Lys Ile Asp Gly Lys Arg Arg Ala Thr Gln
    65      70      75      80
40  aac ctg gtc gaa gtc gag gac cca act ggg ctg atg ggc ggc att cat 288
    Asn Leu Val Glu Val Glu Asp Pro Thr Gly Leu Met Gly Gly Ile His
    85      90      95
45  cgc cgc tgc cct aaa aat ccg ctt atc gac gga ccc gcc agc ggt ttt 336
    Arg Arg Ser Pro Lys Asn Pro Leu Ile Asp Gly Pro Ala Ser Gly Phe
    100      105      110
50  acg ccc cac tac cgc gat ccc atg atc agc cct gat ggg gat ggt tgg 384
    Thr Pro His Tyr Arg Asp Pro Met Ile Ser Pro Asp Gly Asp Gly Trp
    115      120      125
55  aag atg gtt ctt ggg gct cag cgc gaa aac ctg acc ggt gca gcg gtt 432
    Lys Met Val Leu Gly Ala Gln Arg Glu Asn Leu Thr Gly Ala Ala Val
    130      135      140
60  cta tac cgc tgc gca gat ctt gaa aac tgg gaa ttc tcc ggt gaa atc 480
    Leu Tyr Arg Ser Ala Asp Leu Glu Asn Trp Glu Phe Ser Gly Glu Ile
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    Thr Phe Asp Leu Ser Asp
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<213> Corynebacterium thermoaminogenes

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 35 40 45
 Gly Cys Tyr Ser Gly Gly Ala Val Phe Ser Asp Gly Thr Leu Lys Leu
 50 55 60
 Phe Tyr Thr Gly Asn Arg Lys Ile Asp Gly Lys Arg Arg Ala Thr Gln
 65 70 75 80
 Asn Leu Val Glu Val Glu Asp Pro Thr Gly Leu Met Gly Gly Ile His
 85 90 95
 Arg Arg Ser Pro Lys Asn Pro Leu Ile Asp Gly Pro Ala Ser Gly Phe
 100 105 110
 Thr Pro His Tyr Arg Asp Pro Met Ile Ser Pro Asp Gly Asp Gly Trp
 115 120 125
 Lys Met Val Leu Gly Ala Gln Arg Glu Asn Leu Thr Gly Ala Ala Val
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 Thr Phe Asp Leu Ser Asp
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 Trp Ala His Thr Thr Thr Pro Leu Thr Gly Pro Gln Arg Leu Gln Trp
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 5 acg cac ctg ccc gac gct ctt tac ccg gat gca tcc tat gac ctg gat 144
 Thr His Leu Pro Asp Ala Leu Tyr Pro Asp Ala Ser Tyr Asp Leu Asp
 35 40 45
 10 gga tgc tat tcc ggt gga gcc gta ttt act gac ggc aca ctt aaa ctt 192
 Gly Cys Tyr Ser Gly Gly Ala Val Phe Thr Asp Gly Thr Leu Lys Leu
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 15 ttc tac acc ggc aac cta aaa att gac ggc aag cgc cgc gcc acc caa 240
 Phe Tyr Thr Gly Asn Leu Lys Ile Asp Gly Lys Arg Arg Ala Thr Gln
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 aac ctg gtc gaa gtc gag gac cca act ggg ctg atg ggc ggc att cat 288
 Asn Leu Val Glu Val Glu Asp Pro Thr Gly Leu Met Gly Gly Ile His
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 Arg Arg Ser Pro Lys Asn Pro Leu Ile Asp Gly Pro Ala Ser Gly Phe
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 25 aca ccc cat tac cgc gat ccc atg atc agc cct gat ggt gat ggt tgg 384
 Thr Pro His Tyr Arg Asp Pro Met Ile Ser Pro Asp Gly Asp Gly Trp
 115 120 125
 30 aaa atg gtt ctt ggg gcc caa cgc gaa aac ctg acc ggt gca gcg gtt 432
 Lys Met Val Leu Gly Ala Gln Arg Glu Asn Leu Thr Gly Ala Ala Val
 130 135 140
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<211> 159

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<213> Corynebacterium thermoaminogenes

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 35 40 45
 Gly Cys Tyr Ser Gly Gly Ala Val Phe Thr Asp Gly Thr Leu Lys Leu
 50 55 60
 55 Phe Tyr Thr Gly Asn Leu Lys Ile Asp Gly Lys Arg Arg Ala Thr Gln

65 70 75 80
 Asn Leu Val Glu Val Glu Asp Pro Thr Gly Leu Met Gly Gly Ile His
 85 90 95
 5 Arg Arg Ser Pro Lys Asn Pro Leu Ile Asp Gly Pro Ala Ser Gly Phe
 100 105 110
 Thr Pro His Tyr Arg Asp Pro Met Ile Ser Pro Asp Gly Asp Gly Trp
 115 120 125
 10 Lys Met Val Leu Gly Ala Gln Arg Glu Asn Leu Thr Gly Ala Ala Val
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 tttagaaccl ggagalgaag aagaaaaatg gtgtgttctc tggtagaggt atagtcгааг 180
 atgataagtl gtatttatll talacaggtc accattatta taatgacgat gatcccgatc 240
 attttlggca aaatcaaaat alggcclata gigaagatgg catlcatlll caaaaatata 300
 30 aacaaaatgc aalcaliccl accccacctg aagataaatc aacacacitc agagalccaa 360
 aggtatggga acatccatgg ctatttatta catgalagta ggtagtcгаа atgalagaga 420
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-<221>-CDS-

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Ala Gln Leu Ser Gly Gly Gln Gln Gln Arg Val Ala Ile Ala Arg Ala
 135 140 145 150
 5 ctc gcg atg aac ccc aag atc atg ctt ttc gac gaa cca acc tcc gcc 1134
 Leu Ala Met Asn Pro Lys Ile Met Leu Phe Asp Glu Pro Thr Ser Ala
 155 160 165
 10 ctc gac ccc gag atg gtc aac gag gtt ctg gac gtc atg gcg agt ctg 1182
 Leu Asp Pro Glu Met Val Asn Glu Val Leu Asp Val Met Ala Ser Leu
 170 175 180
 gcc aag gaa ggc atg acc atg gtc tgc gtc acc cac gag atg ggt ttc 1230
 Ala Lys Glu Gly Met Thr Met Val Cys Val Thr His Glu Met Gly Phe
 15 185 190 195
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 Ala Arg Arg Ala Ala Asp Arg Val Leu Phe Met Ser Asp Gly Ala Ile
 200 205 210
 20 gtc gag gac tcc gac ccg gag acc ttc ttc acc aat cca caa acc gac 1326
 Val Glu Asp Ser Asp Pro Glu Thr Phe Phe Thr Asn Pro Gln Thr Asp
 215 220 225 230
 cgg gcg aag gat ttc ctg ggc aag atc ctc gcc cac tgacctcccc 1372
 25 Arg Ala Lys Asp Phe Leu Gly Lys Ile Leu Ala His
 235 240
 tcactctgtg tccaactccc ccgttggcca aaatcagcga ccatgacca caggagcatt 1432
 a atg tgc cac aaa cgc atg ttc acc cgt ctc gcc gca gcc acc agc gca 1481
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 gct gtt ctc gcc ggc atc acc ctc acc gcc tgc ggt gat tcc gag ggt 1529
 Ala Val Leu Ala Gly Ile Thr Leu Thr Ala Cys Gly Asp Ser Glu Gly
 35 260 265 270
 ggt gac ggt ctg ctc gcc gcc atc gaa aat ggc aat gtc acc atc ggc 1577
 Gly Asp Gly Leu Leu Ala Ala Ile Glu Asn Gly Asn Val Thr Ile Gly
 275 280 285 290
 40 acc aag tac gat cag ccg ggt ctg gga ctg cgt aac ccg gac aat tcc 1625
 Thr Lys Tyr Asp Gln Pro Gly Leu Gly Leu Arg Asn Pro Asp Asn Ser
 295 300 305
 atg agc gga ctg gat gtc gac gtc gcg cag tac gtc gtc aac tcc atc 1673
 45 Met Ser Gly Leu Asp Val Asp Val Ala Gln Tyr Val Val Asn Ser Ile
 310 315 320
 gcc gat gac aac ggt tgg gat cac ccc acc gtc gaa tgg cgc gag acc 1721
 Ala Asp Asp Asn Gly Trp Asp His Pro Thr Val Glu Trp Arg Glu Thr
 50 325 330 335
 ccc tcc gcc cag cgc gag acc ctc atc cag aac ggt gag gtc gat atg 1769
 Pro Ser Ala Gln Arg Glu Thr Leu Ile Gln Asn Gly Glu Val Asp Met
 340 345 350
 55 atc gcc gca acc tac tcc atc aac ccc gga cgc tcc gaa tgc gtc aac 1817

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Ser Ala Ile Gly Ser Met Ile Leu Gly Thr Ile Leu Thr Ala Met Arg
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 Val Ser Pro Val Lys Ile Leu Arg Ser Ile Ser Thr Ala Tyr Ile Asn
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 Thr Val Arg Asn Thr Pro Leu Thr Leu Val Ile Leu Phe Cys Ser Phe
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 Gly Leu Tyr Gln Asn Leu Gly Leu Thr Leu Ala Gly Arg Asp Ser Ser
 15 610 615 620 625
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 Thr Phe Leu Ala Asp Asn Asn Phe Arg Leu Ala Val Leu Gly Phe Ile
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 20 ctg tac acc tcc gcc ttc gtt gcg gaa tca ctc cgg tca gcc atc aac 2794
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 645 650 655
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 Thr Val His Phe Gly Gln Ala Glu Ala Ala Arg Ser Leu Gly Leu Gly
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 Phe Ser Asp Ile Phe Arg Ser Ile Ile Phe Pro Gln Ala Val Arg Ala
 30 675 680 685
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 725 730 735
 ttc gcc gtc ggc ttc atg atc ctc acc ctc ccc atg ggc ctg ggg ctt 3082
 Phe Ala Val Gly Phe Met Ile Leu Thr Leu Pro Met Gly Leu Gly Leu
 45 740 745 750
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 Gly Lys Leu Ala Glu Lys Met Ala Val Lys Lys
 755 760
 50 gcaacagtc tctacgacgc ccccgcccc cggggacgca ggccaacac catcatcacc 3195
 atgccacca ccttggtggc agtggcgc cigtctgg gig ggc agt gtt ctc 3249
 Val Gly Ser Val Leu
 765
 55 cag gaa aac ggc cag ttg gac ggc gac aaa tgg acc ccg ttc ctc gat 3297

	Gln	Glu	Asn	Gly	Gln	Leu	Asp	Gly	Asp	Lys	Trp	Thr	Pro	Phe	Leu	Asp	
	770					775					780					785	
5	ccc	cag	acc	igg	acc	acc	tat	ctt	ctg	ccc	ggc	ctg	igg	gga	acc	ctg	3345
	Pro	Gln	Thr	Trp	Thr	Thr	Tyr	Leu	Leu	Pro	Gly	Leu	Trp	Gly	Thr	Leu	
					790					795					800		
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	Lys	Ala	Ala	Val	Ala	Ser	Ile	Leu	Leu	Ala	Leu	Ile	Met	Gly	Thr	Leu	
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	Leu	Gly	Leu	Gly	Arg	Ile	Ser	Glu	Ile	Arg	Leu	Leu	Arg	Trp	Phe	Cys	
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	Gly	Ile	Ile	Ile	Glu	Thr	Phe	Arg	Ala	Ile	Pro	Val	Leu	Ile	Leu	Met	
		835					840				845						
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	Ile	Phe	Ala	Tyr	Gln	Leu	Phe	Ala	Arg	Tyr	Gln	Leu	Val	Pro	Ser	Arg	
	850				855					860					865		
30	cag	ctg	gcc	ttc	gcc	gcg	gig	gtc	ttc	ggt	ctc	acc	atg	tac	aac	ggc	3585
	Gln	Leu	Ala	Phe	Ala	Ala	Val	Val	Phe	Gly	Leu	Thr	Met	Tyr	Asn	Gly	
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	Ser	Val	Ile	Ala	Glu	Ile	Leu	Arg	Ser	Gly	Ile	Ala	Ser	Leu	Pro	Lys	
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	Gly	Gln	Arg	Glu	Ala	Ala	Ile	Ala	Leu	Gly	Met	Ser	Thr	Arg	Gln	Thr	
		900						905					910				
45	acc	igg	tcc	atc	ctg	ctc	ccc	cag	gcg	gig	gca	gcg	atg	ctg	ccc	gcc	3729
	Thr	Trp	Ser	Ile	Leu	Leu	Pro	Gln	Ala	Val	Ala	Ala	Met	Leu	Pro	Ala	
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	Leu	Ile	Ala	Gln	Met	Val	Ile	Ala	Leu	Lys	Asp	Ser	Ala	Leu	Gly	Tyr	
	930					935				940				945			
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	Gln	Ile	Gly	Tyr	Ile	Glu	Val	Val	Arg	Ser	Gly	Ile	Gln	Ser	Ala	Ser	
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	Val	Asn	Arg	Asn	Tyr	Leu	Ala	Ala	Leu	Ala	Val	Val	Ala	Val	Ile	Met	
				965				970					975				
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	Ile	Leu	Ile	Asn	Phe	Ala	Leu	Thr	Ala	Leu	Ala	Glu	Arg	Ile	Gln	Arg	
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	Gln	Leu	Arg	Ala	Gly	Arg	Ala	Arg	Arg	Asn	Ile	Val	Ala	Lys	Val	Pro	

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 Trp His Asp Pro Asp Tyr Lys Glu Val Lys His Pro Gly Pro Ser Phe
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 Arg Leu Glu Thr Ile Glu Glu Gly Thr Ile Glu Ile Asp Gly Lys Leu
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 Leu Pro Glu Glu Gly Lys Asp Leu Ala Lys Ile Arg Ala Asp Val Gly
 65 70 75 80
 Met Val Phe Gln Ser Phe Asn Leu Phe Pro His Leu Thr Ile Lys Asp
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 Asn Val Thr Leu Gly Pro Met Lys Val Arg Lys Met Lys Lys Ser Glu
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 Ala Asn Glu Val Ala Met Lys Leu Glu Arg Val Gly Ile Ala Asn
 115 120 125
 Gln Ala Glu Lys Tyr Pro Ala Gln Leu Ser Gly Gly Gln Gln Gln Arg
 130 135 140
 Val Ala Ile Ala Arg Ala Leu Ala Met Asn Pro Lys Ile Met Leu Phe
 145 150 155 160
 Asp Glu Pro Thr Ser Ala Leu Asp Pro Glu Met Val Asn Glu Val Leu
 165 170 175
 Asp Val Met Ala Ser Leu Ala Lys Glu Gly Met Thr Met Val Cys Val
 180 185 190
 Thr His Glu Met Gly Phe Ala Arg Arg Ala Ala Asp Arg Val Leu Phe

195 200 205
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 <213> *Corynebacterium thermoaminogenes*
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 20 25 30
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 25 35 40 45
 Thr Lys Tyr Asp Gln Pro Gly Leu Gly Leu Arg Asn Pro Asp Asn Ser
 50 55 60
 Met Ser Gly Leu Asp Val Asp Val Ala Gln Tyr Val Val Asn Ser Ile
 30 65 70 75 80
 Ala Asp Asp Asn Gly Trp Asp His Pro Thr Val Glu Trp Arg Glu Thr
 85 90 95
 Pro Ser Ala Gln Arg Glu Thr Leu Ile Gln Asn Gly Glu Val Asp Met
 100 105 110
 Ile Ala Ala Thr Tyr Ser Ile Asn Pro Gly Arg Ser Glu Ser Val Asn
 115 120 125
 Phe Gly Gly Pro Tyr Leu Leu Thr His Gln Ala Leu Leu Val Arg Glu
 40 130 135 140
 Asp Asp Asp Arg Ile Gln Thr Leu Glu Asp Leu Asp Asp Gly Leu Ile
 145 150 155 160
 Leu Cys Ser Val Thr Gly Ser Thr Pro Ala Gln Lys Val Lys Asp Val
 45 165 170 175
 Leu Pro Gly Val Gln Leu Gln Glu Tyr Asp Thr Tyr Ser Ser Cys Val
 180 185 190
 Glu Ala Leu Ser Gln Gly Asn Val Asp Ala Met Thr Thr Asp Ala Thr
 50 195 200 205
 Ile Leu Phe Gly Tyr Ala Gln Gln Arg Glu Gly Glu Phe Arg Val Val
 210 215 220
 Glu Met Glu Gln Asp Gly Glu Pro Phe Thr Asn Glu Tyr Tyr Gly Ile
 55 225 230 235 240

Gly Ile Thr Lys Asp Asp Thr Glu Ala Thr Asp Ala Ile Asn Ala Ala
 245 250 255
 5 Leu Glu Arg Met Tyr Ala Asp Gly Ser Phe Gln Arg Phe Leu Thr Glu
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 Asn Leu Gly Glu Asp Ser Gln Val Val Gln Glu Gly Thr Pro Gly Asp
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 10 Leu Ser Phe Leu Asp Glu
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<212> PRT

<213> *Corynebacterium thermoaminogenes*

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 25 Leu Gly Thr Ile Leu Thr Ala Met Arg Val Ser Pro Val Lys Ile Leu
 35 40 45
 Arg Ser Ile Ser Thr Ala Tyr Ile Asn Thr Val Arg Asn Thr Pro Leu
 30 50 55 60
 Thr Leu Val Ile Leu Phe Cys Ser Phe Gly Leu Tyr Gln Asn Leu Gly
 65 70 75 80
 Leu Thr Leu Ala Gly Arg Asp Ser Ser Thr Phe Leu Ala Asp Asn Asn
 35 85 90 95
 Phe Arg Leu Ala Val Leu Gly Phe Ile Leu Tyr Thr Ser Ala Phe Val
 100 105 110
 Ala Glu Ser Leu Arg Ser Gly Ile Asn Thr Val His Phe Gly Gln Ala
 40 115 120 125
 Glu Ala Ala Arg Ser Leu Gly Leu Gly Phe Ser Asp Ile Phe Arg Ser
 130 135 140
 Ile Ile Phe Pro Gln Ala Val Arg Ala Ala Ile Ile Pro Leu Gly Asn
 45 145 150 155 160
 Thr Leu Ile Ala Leu Thr Lys Asn Thr Thr Ile Ala Ser Val Ile Gly
 165 170 175
 Val Gly Glu Ala Ser Leu Leu Met Lys Ser Thr Ile Glu Asn His Ala
 50 180 185 190
 Asn Met Leu Phe Val Val Phe Ala Ile Phe Ala Val Gly Phe Met Ile
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 55 Leu Thr Leu Pro Met Gly Leu Gly Leu Gly Lys Leu Ala Glu Lys Met
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Ala Val Lys Lys

225

<210> 20

<211> 277

<212> PRT

<213> Corynebacterium thermoaminogenes

<400> 20

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 35 40 45
 Ile Met Gly Thr Leu Leu Gly Leu Gly Arg Ile Ser Glu Ile Arg Leu
 50 55 60
 Leu Arg Trp Phe Cys Gly Ile Ile Ile Glu Thr Phe Arg Ala Ile Pro
 65 70 75 80
 Val Leu Ile Leu Met Ile Phe Ala Tyr Gln Leu Phe Ala Arg Tyr Gln
 85 90 95
 Leu Val Pro Ser Arg Gln Leu Ala Phe Ala Ala Val Val Phe Gly Leu
 100 105 110
 Thr Met Tyr Asn Gly Ser Val Ile Ala Glu Ile Leu Arg Ser Gly Ile
 115 120 125
 Ala Ser Leu Pro Lys Gly Gln Arg Glu Ala Ala Ile Ala Leu Gly Met
 130 135 140
 Ser Thr Arg Gln Thr Thr Trp Ser Ile Leu Leu Pro Gln Ala Val Ala
 145 150 155 160
 Ala Met Leu Pro Ala Leu Ile Ala Gln Met Val Ile Ala Leu Lys Asp
 165 170 175
 Ser Ala Leu Gly Tyr Gln Ile Gly Tyr Ile Glu Val Val Arg Ser Gly
 180 185 190
 Ile Gln Ser Ala Ser Val Asn Arg Asn Tyr Leu Ala Ala Leu Ala Val
 195 200 205
 Val Ala Val Ile Met Ile Leu Ile Asn Phe Ala Leu Thr Ala Leu Ala
 210 215 220
 Glu Arg Ile Gln Arg Gln Leu Arg Ala Gly Arg Ala Arg Arg Asn Ile
 225 230 235 240
 Val Ala Lys Val Pro Glu Glu Pro Asp Gln Gly Leu Asp Thr Lys Asp
 245 250 255
 Asn Val Asn Val Asp Trp His Asp Pro Asp Tyr Lys Glu Val Lys His
 260 265 270

Pro Gly Pro Ser Phe
275

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<210> 21

<211> 3598

<212> DNA

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<213> *Corynebacterium thermoaminogenes*

<220>

<221> CDS

<222> (454).. (3222)

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atcagcaggt gaaacagacc ctcttgaat gctttgttaa aaagaaccgc cctttgtgcg 240
tatctttgtg tcaattgtgc gcgcacigcc accagcttct ctccaggatg aacacggctg 300
ggaaatccct cccggalacc ctgcacgccc caccctccac accgacaccg gcggggaggg 360
ccgggcacgt tticagctgc gggatgaagga agcggctgcc ggtccccggg tcgcataaac 420
gaaatgaaaa acattccaac aggagggtg gaa atg gcc gat caa gca aaa ctt 474

Met Ala Asp Gln Ala Lys Leu

1

5

30

ggt ggc aaa ccc aca gat gac acc aac ttc gcg atg atc cgt gat ggc 522
Gly Gly Lys Pro Thr Asp Asp Thr Asn Phe Ala Met Ile Arg Asp Gly

10

15

20

35

gtt gca tct tat ttg aac gac tcc gac ccg gag gag acc aag gag tgg 570
Val Ala Ser Tyr Leu Asn Asp Ser Asp Pro Glu Glu Thr Lys Glu Trp

25

30

35

40

atg gac tcc cta gac ggt cta ctg cag gat tcc tct ccg gag cgc gcc 618
Met Asp Ser Leu Asp Gly Leu Leu Gln Asp Ser Ser Pro Glu Arg Ala
40 45 50 55
cgt tac ctg atg ctg cgc ctg ctg gag cgg gca tcc gcc aag cgt gtc 666
Arg Tyr Leu Met Leu Arg Leu Leu Glu Arg Ala Ser Ala Lys Arg Val

60

65

70

45

cca ctg ccc ccg atg acg tcc acc gat tac gtc aac acc atc ccc aca 714
Pro Leu Pro Pro Met Thr Ser Thr Asp Tyr Val Asn Thr Ile Pro Thr

75

80

85

50

tcc atg gag ccc gat ttc ccg ggt gat gag gag atg gag aag cgc tac 762
Ser Met Glu Pro Asp Phe Pro Gly Asp Glu Glu Met Glu Lys Arg Tyr

90

95

100

55

cgc cgc tgg atg cgc tgg aac gcc gcc atc atg gtg cac cgt gcc cag 810
Arg Arg Trp Met Arg Trp Asn Ala Ala Ile Met Val His Arg Ala Gln
105 110 115
cgc ccg gga atc ggt gtg ggt ggg cac atc tcc acc tac gcc ggc gcc 858

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	Ala	Pro	Leu	Tyr	Glu	Val	Gly	Phe	Asn	His	Phe	Phe	Arg	Gly	Lys	Asp	
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	ggc	atg	tac	gcc	cgc	gcc	ttc	ctc	gag	ggc	cgt	ctc	acc	gag	agc	gat	1002
	Gly	Met	Tyr	Ala	Arg	Ala	Phe	Leu	Glu	Gly	Arg	Leu	Thr	Glu	Ser	Asp	
15					170					175					180		
	ctg	gac	agc	ttc	cgc	cag	gag	gtc	ttc	tac	gaa	ggt	ggt	ggc	atc	ccg	1050
	Leu	Asp	Ser	Phe	Arg	Gln	Glu	Val	Ser	Tyr	Glu	Gly	Gly	Gly	Ile	Pro	
					185					190					195		
20	ttc	tac	ccg	cac	ccg	cac	ggc	atg	ccg	gac	ttc	igg	gag	ttc	ccg	acc	1098
	Ser	Tyr	Pro	His	Pro	His	Gly	Met	Pro	Asp	Phe	Trp	Glu	Phe	Pro	Thr	
	200									205					210		
	gtg	ttc	atg	ggc	ctc	ggg	ccc	atg	gat	gcc	atc	tac	cag	gcg	cgc	ttc	1146
25	Val	Ser	Met	Gly	Leu	Gly	Pro	Met	Asp	Ala	Ile	Tyr	Gln	Ala	Arg	Phe	
					220					225					230		
	aac	cgc	tac	ctg	cac	aac	cgt	ggc	atc	aag	gac	acc	tcg	gag	cag	cac	1194
	Asn	Arg	Tyr	Leu	His	Asn	Arg	Gly	Ile	Lys	Asp	Thr	Ser	Glu	Gln	His	
30					235					240					245		
	gtc	igg	gca	ttc	ctc	ggt	gac	ggc	gag	atg	gat	gag	ccg	gag	ttc	cgt	1242
	Val	Trp	Ala	Phe	Leu	Gly	Asp	Gly	Glu	Met	Asp	Glu	Pro	Glu	Ser	Arg	
					250					255					260		
35	ggt	ctc	atc	cac	cag	gct	gcg	ctg	aac	aac	ctg	gac	aac	ctc	acc	ttc	1290
	Gly	Leu	Ile	His	Gln	Ala	Ala	Leu	Asn	Asn	Leu	Asp	Asn	Leu	Thr	Phe	
					265					270					275		
	gtg	atc	aac	tcg	aac	ctg	cag	cgt	ctt	gat	ggc	ccg	gtc	cgc	ggt	aac	1338
40	Val	Ile	Asn	Cys	Asn	Leu	Gln	Arg	Leu	Asp	Gly	Pro	Val	Arg	Gly	Asn	
	280									285					290		
	acc	aag	atc	atc	cag	gaa	ctc	gag	ttc	ttc	ttc	cgt	ggt	gcc	ggc	ttg	1386
	Thr	Lys	Ile	Ile	Gln	Glu	Leu	Glu	Ser	Phe	Phe	Arg	Gly	Ala	Gly	Trp	
45					300					305					310		
	ttc	gtc	atc	aag	gtc	atc	ttg	ggc	cgt	gag	ttg	gat	gaa	ctg	ctg	gag	1434
	Ser	Val	Ile	Lys	Val	Ile	Trp	Gly	Arg	Glu	Trp	Asp	Glu	Leu	Leu	Glu	
					315					320					325		
50	aag	gac	cag	gac	ggt	gct	ctt	gtc	gag	gtc	atg	aac	aac	acc	ttc	gac	1482
	Lys	Asp	Gln	Asp	Gly	Ala	Leu	Val	Glu	Val	Met	Asn	Asn	Thr	Ser	Asp	
					330					335					340		
55	ggt	gac	tac	cag	acc	ttc	aag	gcc	aat	gac	ggt	gcc	tac	gtc	cgt	gag	1530
	Gly	Asp	Tyr	Gln	Thr	Phe	Lys	Ala	Asn	Asp	Gly	Ala	Tyr	Val	Arg	Glu	

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	cac ttc ttc ggc cgt gac ccc cgc acc ctc aag ctc gtc gag gac atg			1578
5	His Phe Phe Gly Arg Asp Pro Arg Thr Leu Lys Leu Val Glu Asp Met			
	360	365	370	375
	acc gac gag gag atc tgg aag ctg ccc cgt ggt ggc cal gac tac cgt			1626
	Thr Asp Glu Glu Ile Trp Lys Leu Pro Arg Gly Gly His Asp Tyr Arg			
10		380	385	390
	aag gtc tac gcc gcc tac aag cgt gcg ctg gag acc aag gac cgc ccg			1674
	Lys Val Tyr Ala Ala Tyr Lys Arg Ala Leu Glu Thr Lys Asp Arg Pro			
	395	400	405	
15	acc gtc att ctc gcc cal acc atc aag ggc tac ggc ctg ggc cac aac			1722
	Thr Val Ile Leu Ala His Thr Ile Lys Gly Tyr Gly Leu Gly His Asn			
	410	415	420	
	ttc gag ggc cgc aac gcg acc cac cag atg aag aag ctg acc ctg gat			1770
20	Phe Glu Gly Arg Asn Ala Thr His Gln Met Lys Lys Leu Thr Leu Asp			
	425	430	435	
	gac ctg aag ctg ttc cgt gac aag cag ggt ctg ccc atc acc gat gag			1818
	Asp Leu Lys Leu Phe Arg Asp Lys Gln Gly Leu Pro Ile Thr Asp Glu			
25	440	445	450	455
	gag ctg gag aag gat ccc tac ctg cct ccg tac tac cac ccg ggt gag			1866
	Glu Leu Glu Lys Asp Pro Tyr Leu Pro Pro Tyr Tyr His Pro Gly Glu			
	460	465	470	
30	gac gca ccg gag atc aag tac atg aag gag cgt cgc cag gcg ctc ggt			1914
	Asp Ala Pro Glu Ile Lys Tyr Met Lys Glu Arg Arg Gln Ala Leu Gly			
	475	480	485	
	ggt ttc ctg ccg gag cgc cgt gag aag tac gag cca ctg cag gtt ccc			1962
35	Gly Phe Leu Pro Glu Arg Arg Glu Lys Tyr Glu Pro Leu Gln Val Pro			
	490	495	500	
	ccg ctg gac aag ctg cgg tcc gtg cgc aag ggt tcc ggc aag cag cag			2010
	Pro Leu Asp Lys Leu Arg Ser Val Arg Lys Gly Ser Gly Lys Gln Gln			
40	505	510	515	
	gtg gcc acc acc atg gcc acg gtg cgt acc ttc aag gaa ctc atg cgg			2058
	Val Ala Thr Thr Met Ala Thr Val Arg Thr Phe Lys Glu Leu Met Arg			
	520	525	530	535
45	gac aag aac ctg gcc gac cgc ttg gtc ccg atc atc ccg gat gag gcc			2106
	Asp Lys Asn Leu Ala Asp Arg Leu Val Pro Ile Ile Pro Asp Glu Ala			
	540	545	550	
	cgc acc ttc ggc ctg gac tcc tgg ttc ccg acc ctg aaa atc tac aac			2154
50	Arg Thr Phe Gly Leu Asp Ser Trp Phe Pro Thr Leu Lys Ile Tyr Asn			
	555	560	565	
	ccg cac ggt cag aac tac gtg ccg gtc gac cal gac ctc atg ctg tcc			2202
	Pro His Gly Gln Asn Tyr Val Pro Val Asp His Asp Leu Met Leu Ser			
55	570	575	580	

	lac cgl gag gcc aag gac ggc cag atc ctg cal gag ggc atc aac gag	2250
	Tyr Arg Glu Ala Lys Asp Gly Gln Ile Leu His Glu Gly Ile Asn Glu	
5	585 590 595	
	gcc ggt tcc gtg gca tgc ttt atc gcc gcc gga acc tcc tac gcc acc	2298
	Ala Gly Ser Val Ala Ser Phe Ile Ala Ala Gly Thr Ser Tyr Ala Thr	
	600 605 610 615	
10	cat ggc gag gcc atg atc ccg ctg tac atc ttc tac tgc atg ttc ggc	2346
	His Gly Glu Ala Met Ile Pro Leu Tyr Ile Phe Tyr Ser Met Phe Gly	
	620 625 630	
	ttc cag cgc acc ggt gac ggc atc tgg gcc gca gcc gac cag atg acg	2394
15	Phe Gln Arg Thr Gly Asp Gly Ile Trp Ala Ala Ala Asp Gln Met Thr	
	635 640 645	
	cgt ggt ttc ctg ctg ggc gcc acc gcc ggt cgc acc acc ctg acc ggt	2442
	Arg Gly Phe Leu Leu Gly Ala Thr Ala Gly Arg Thr Thr Leu Thr Gly	
20	650 655 660	
	gag gcc ctg cag cac atg gat ggc cac tcc ccg atc ctg gcc tcc acc	2490
	Glu Gly Leu Gln His Met Asp Gly His Ser Pro Ile Leu Ala Ser Thr	
	665 670 675	
25	aac ccc ggt gtg gag acc tat gac ccg gcg ttc tcc tac gag atc gcg	2538
	Asn Pro Gly Val Glu Thr Tyr Asp Pro Ala Phe Ser Tyr Glu Ile Ala	
	680 685 690 695	
	cac ctg gtc cac cgc ggc atc gac cgc atg tac gga ccg ggc aag ggt	2586
30	His Leu Val His Arg Gly Ile Asp Arg Met Tyr Gly Pro Gly Lys Gly	
	700 705 710	
	gag aat gtc atc tac tac ctg acc atc lac aac gag cca acc ccg cag	2634
	Glu Asn Val Ile Tyr Tyr Leu Thr Ile Tyr Asn Glu Pro Thr Pro Gln	
35	715 720 725	
	ccg gct gag cct gag gat ctg gac gtc gag ggc ctg cac aag ggc atc	2682
	Pro Ala Glu Pro Glu Asp Leu Asp Val Glu Gly Leu His Lys Gly Ile	
	730 735 740	
40	tac ctg tac gac aag gcc gcc gag ggt gag ggc cal gag gcc tgc atc	2730
	Tyr Leu Tyr Asp Lys Ala Ala Glu Gly Glu Gly His Glu Ala Ser Ile	
	745 750 755	
	ctg gcc tcc ggc atc ggc atg cag tgg gca ctg cgc gcc cgt gac atc	2778
45	Leu Ala Ser Gly Ile Gly Met Gln Trp Ala Leu Arg Ala Arg Asp Ile	
	760 765 770 775	
	ctc gcc gag gat tac ggc atc cgt gcc aac atc ttc tcc gcc acc tgc	2826
	Leu Ala Glu Asp Tyr Gly Ile Arg Ala Asn Ile Phe Ser Ala Thr Ser	
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	tgg gtg gag ctg gcc cgc gac ggt gcc cgc cgt aac ctg gag gcg ctg	2874
	Trp Val Glu Leu Ala Arg Asp Gly Ala Arg Arg Asn Leu Glu Ala Leu	
	795 800 805	
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Arg Asn Pro Gly Ala Asp Val Gly Glu Ala Phe Val Thr Thr Gln Leu
 810 815 820
 5 aag aag ggt tcc ggc ccc tac gtc gcg glg tcc gac ttc gcg acc gac 2970
 Lys Lys Gly Ser Gly Pro Tyr Val Ala Val Ser Asp Phe Ala Thr Asp
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 10 ctg ccg aac cag atc cgc gag tgg gtt ccc ggt gac tac atc gtc ctc 3018
 Leu Pro Asn Gln Ile Arg Glu Trp Val Pro Gly Asp Tyr Ile Val Leu
 840 845 850 855
 ggt gcc gac ggc ttc ggt ttc tcc gat acc cgt ccg gca gcc cgt cgt 3066
 Gly Ala Asp Gly Phe Gly Phe Ser Asp Thr Arg Pro Ala Ala Arg Arg
 15 860 865 870
 tac ttc aac atc gac gcc gag tcc atc gtc glg gcg gtc ctg cgc ggc 3114
 Tyr Phe Asn Ile Asp Ala Glu Ser Ile Val Val Ala Val Leu Arg Gly
 875 880 885
 20 ctg gtc cgc gag ggt gtc atc gat gcc tcc glg gcg gcg cac gcg gct 3162
 Leu Val Arg Glu Gly Val Ile Asp Ala Ser Val Ala Ala His Ala Ala
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 gag aag tac aag ctg tcc gac ccg acg gca cca cag gtc gat ccg gac 3210
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 905 910 915
 gca ccg atc gag tagaccigt tgcgcagaa aaacaccccc gcccccctac 3262
 Ala Pro Ile Glu
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 <213> *Corynebacterium thermoaminogenes*
 45
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 50 Phe Ala Met Ile Arg Asp Gly Val Ala Ser Tyr Leu Asn Asp Ser Asp
 20 25 30
 Pro Glu Glu Thr Lys Glu Trp Met Asp Ser Leu Asp Gly Leu Leu Gln
 35 40 45
 55 Asp Ser Ser Pro Glu Arg Ala Arg Tyr Leu Met Leu Arg Leu Leu Glu

	50		55		60											
	Arg	Ala	Ser	Ala	Lys	Arg	Val	Pro	Leu	Pro	Pro	Met	Thr	Ser	Thr	Asp
	65					70						75				80
5	Tyr	Val	Asn	Thr	Ile	Pro	Thr	Ser	Met	Glu	Pro	Asp	Phe	Pro	Gly	Asp
					85					90					95	
	Glu	Glu	Met	Glu	Lys	Arg	Tyr	Arg	Arg	Trp	Met	Arg	Trp	Asn	Ala	Ala
				100					105					110		
10	Ile	Met	Val	His	Arg	Ala	Gln	Arg	Pro	Gly	Ile	Gly	Val	Gly	Gly	His
				115				120					125			
	Ile	Ser	Thr	Tyr	Ala	Gly	Ala	Ala	Pro	Leu	Tyr	Glu	Val	Gly	Phe	Asn
				130				135					140			
15	His	Phe	Phe	Arg	Gly	Lys	Asp	His	Pro	Gly	Gly	Gly	Asp	Gln	Val	Phe
	145					150					155					160
	Phe	Gln	Gly	His	Ala	Ser	Pro	Gly	Met	Tyr	Ala	Arg	Ala	Phe	Leu	Glu
				165						170						175
20	Gly	Arg	Leu	Thr	Glu	Ser	Asp	Leu	Asp	Ser	Phe	Arg	Gln	Glu	Val	Ser
				180					185					190		
	Tyr	Glu	Gly	Gly	Gly	Ile	Pro	Ser	Tyr	Pro	His	Pro	His	Gly	Met	Pro
				195				200					205			
25	Asp	Phe	Trp	Glu	Phe	Pro	Thr	Val	Ser	Met	Gly	Leu	Gly	Pro	Met	Asp
				210				215				220				
	Ala	Ile	Tyr	Gln	Ala	Arg	Phe	Asn	Arg	Tyr	Leu	His	Asn	Arg	Gly	Ile
	225					230					235					240
30	Lys	Asp	Thr	Ser	Glu	Gln	His	Val	Trp	Ala	Phe	Leu	Gly	Asp	Gly	Glu
				245						250					255	
	Met	Asp	Glu	Pro	Glu	Ser	Arg	Gly	Leu	Ile	His	Gln	Ala	Ala	Leu	Asn
				260				265						270		
35	Asn	Leu	Asp	Asn	Leu	Thr	Phe	Val	Ile	Asn	Cys	Asn	Leu	Gln	Arg	Leu
				275				280					285			
	Asp	Gly	Pro	Val	Arg	Gly	Asn	Thr	Lys	Ile	Ile	Gln	Glu	Leu	Glu	Ser
				290				295				300				
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	305					310					315					320
	Glu	Trp	Asp	Glu	Leu	Glu	Lys	Asp	Gln	Asp	Gly	Ala	Leu	Val	Glu	
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45	Val	Met	Asn	Asn	Thr	Ser	Asp	Gly	Asp	Tyr	Gln	Thr	Phe	Lys	Ala	Asn
				340					345					350		
	Asp	Gly	Ala	Tyr	Val	Arg	Glu	His	Phe	Phe	Gly	Arg	Asp	Pro	Arg	Thr
				355				360					365			
50	Leu	Lys	Leu	Val	Glu	Asp	Met	Thr	Asp	Glu	Glu	Ile	Trp	Lys	Leu	Pro
				370				375				380				
	Arg	Gly	Gly	His	Asp	Tyr	Arg	Lys	Val	Tyr	Ala	Tyr	Lys	Arg	Ala	
55				385				390				395				400

Leu Glu Thr Lys Asp Arg Pro Thr Val Ile Leu Ala His Thr Ile Lys
 405 410 415
 5 Gly Tyr Gly Leu Gly His Asn Phe Glu Gly Arg Asn Ala Thr His Gln
 420 425 430
 Met Lys Lys Leu Thr Leu Asp Asp Leu Lys Leu Phe Arg Asp Lys Gln
 435 440 445
 10 Gly Leu Pro Ile Thr Asp Glu Glu Leu Glu Lys Asp Pro Tyr Leu Pro
 450 455 460
 Pro Tyr Tyr His Pro Gly Glu Asp Ala Pro Glu Ile Lys Tyr Met Lys
 465 470 475 480
 15 Glu Arg Arg Gln Ala Leu Gly Gly Phe Leu Pro Glu Arg Arg Glu Lys
 485 490 495
 Tyr Glu Pro Leu Gln Val Pro Pro Leu Asp Lys Leu Arg Ser Val Arg
 500 505 510
 20 Lys Gly Ser Gly Lys Gln Gln Val Ala Thr Thr Met Ala Thr Val Arg
 515 520 525
 Thr Phe Lys Glu Leu Met Arg Asp Lys Asn Leu Ala Asp Arg Leu Val
 530 535 540
 25 Pro Ile Ile Pro Asp Glu Ala Arg Thr Phe Gly Leu Asp Ser Trp Phe
 545 550 555 560
 Pro Thr Leu Lys Ile Tyr Asn Pro His Gly Gln Asn Tyr Val Pro Val
 565 570 575
 30 Asp His Asp Leu Met Leu Ser Tyr Arg Glu Ala Lys Asp Gly Gln Ile
 580 585 590
 Leu His Glu Gly Ile Asn Glu Ala Gly Ser Val Ala Ser Phe Ile Ala
 595 600 605
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 55 Glu Gly Leu His Lys Gly Ile Tyr Leu Tyr Asp Lys Ala Ala Glu Gly

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 Glu Gly His Glu Ala Ser Ile Leu Ala Ser Gly Ile Gly Met Gln Trp
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 Pro Gly Asp Tyr Ile Val Leu Gly Ala Asp Gly Phe Gly Phe Ser Asp
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 Val Val Thr Thr Thr Pro Ser Thr Leu Pro Ala

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	Phe	Arg	Ala	Ala	Tyr	Glu	Thr	Gly	Ala	Ala	Thr	Val	Ala	Ile	Tyr	Pro	
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	Arg	Ile	Gly	Thr	Glu	Gly	Ser	Pro	Val	Lys	Ala	Tyr	Leu	Asp	Ile	Asp	
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	Glu	Ile	Ile	Asn	Ala	Ala	Lys	Lys	Val	Lys	Ala	Asp	Ala	Val	Tyr	Pro	
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	Pro	Val	Leu	Ala	Glu	Ser	Thr	Pro	Ser	Thr	Asp	Ile	Asp	Glu	Ile	Val	
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	Lys	Ser	Ala	Glu	Gly	Gln	Thr	Tyr	Pro	Ile	Phe	Val	Lys	Ala	Val	Ala	
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40	Gly	Gly	Gly	Gly	Arg	Gly	Met	Arg	Phe	Val	Glu	Lys	Pro	Glu	Asp	Leu	
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	Arg	Glu	Leu	Ala	Arg	Glu	Ala	Ser	Arg	Glu	Ala	Glu	Ala	Ala	Phe	Gly	
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	Glu	Val	Gln	Ile	Leu	Gly	Asp	His	Thr	Gly	Asp	Val	Ile	His	Leu	Tyr	
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25	gtc gac ctg gtc aag gcg cag atg cac ctg gcc gcc ggt gcc acc ctg	1311		
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	Lys Glu Leu Gly Leu Thr Gln Asp Lys Ile Thr Thr His Gly Ala Ala			
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	Leu Gln Cys Arg Ile Thr Thr Glu Asp Pro Ser Asn Asn Phe Arg Pro			
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	Asp Thr Gly Val Ile Thr Ala Tyr Arg Ser Pro Gly Gly Ala Gly Val			
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	Arg Leu Asp Gly Ala Ala Gln Leu Gly Gly Glu Ile Thr Ala His Phe			
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50	gat tcc atg ctg gtc aag atg acc tgc cgc ggt tcc gat ttc gag acc	1551		
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	Val Ala Thr Asn Ile Gly Phe Leu Arg Ala Leu Leu Arg Glu Glu Asp			
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	Leu Leu Gln Ala Pro Pro Ala Asp Asp Glu Gln Gly Arg Ile Leu Glu			
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	Tyr Leu Ala Asp Val Thr Val Asn Lys Pro His Gly Glu Arg Pro Glu	
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	Thr Ala Arg Pro Ile Glu Lys Leu Pro Glu Val Glu Asn Ile Pro Leu	
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15	Ala Arg Asp Leu Arg Glu Gln Asp Ala Leu Ala Val Thr Asp Thr Thr	
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	Ala Leu Thr Pro Ala Ala Arg Ala Val Ala Lys Leu Thr Pro Glu Leu	
	560 565 570	
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	Leu Ser Val Glu Ala Trp Gly Gly Ala Thr Tyr Asp Val Ala Met Arg	
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	Met Pro Asn Val Asn Ile Gln Met Leu Leu Arg Gly Arg Asn Thr Val	
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	Thr Ser Val Ala Glu Val Ala Met Ala Tyr Ser Gly Asp Leu Ser Asn	
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Gly Ser Pro Val Lys Ala Tyr Leu Asp Ile Asp Glu Ile Ile Asn Ala
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Ser Glu Asn Ala Gln Leu Ala Arg Glu Cys Ala Glu Asn Gly Ile Thr
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Gln Thr Tyr Pro Ile Phe Val Lys Ala Val Ala Gly Gly Gly Gly Arg
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225 230 235 240
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Leu Asp Pro Glu Leu Arg Asp Arg Ile Cys Ala Asp Ala Val Lys Phe
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Cys Lys Ser Ile Gly Tyr Gln Gly Ala Gly Thr Val Glu Phe Leu Val

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 Ala Gln Leu Gly Gly Glu Ile Thr Ala His Phe Asp Ser Met Leu Val
 385 390 395 400
 Lys Met Thr Cys Arg Gly Ser Asp Phe Glu Thr Ala Val Ser Arg Ala
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 Gln Arg Ala Leu Ala Glu Phe Asn Val Ser Gly Val Ala Thr Asn Ile
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 Gly Phe Leu Arg Ala Leu Leu Arg Glu Glu Asp Phe Thr Lys Arg Arg
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 Ile Asp Thr Gly Phe Ile Gly Ser His Gln His Leu Leu Gln Ala Pro
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 Pro Ala Asp Asp Glu Gln Gly Arg Ile Leu Glu Tyr Leu Ala Asp Val
 465 470 475 480
 Thr Val Asn Lys Pro His Gly Glu Arg Pro Glu Thr Ala Arg Pro Ile
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 Glu Lys Leu Pro Glu Val Glu Asn Ile Pro Leu Pro Arg Gly Ser Arg
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 Glu Gln Asp Ala Leu Ala Val Thr Asp Thr Thr Phe Arg Asp Ala His
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 Ala Arg Ala Val Ala Lys Leu Thr Pro Glu Leu Leu Ser Val Glu Ala
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 Ile Gln Met Leu Leu Arg Gly Arg Asn Thr Val Gly Tyr Thr Pro Tyr
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	Tyr	Thr	Leu	Asp	Tyr	Tyr	Leu	Asn	Leu	Ala	Glu	Gln	Ile	Val	Asp	Ser	
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				805						810					815		
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				820					825					830			
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			835						840				845				
	Leu	Gly	Leu	Ala	Asp	Arg	Phe	Glu	Leu	Ile	Glu	Asp	Tyr	Tyr	Ala	Ala	
			850				855					860					
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						870					875					880	
	Val	Val	Gly	Asp	Leu	Ala	Leu	His	Leu	Val	Gly	Ala	Gly	Val	Ser	Pro	
				885						890					895		
45	Glu	Asp	Phe	Ala	Ala	Asp	Pro	Gln	Lys	Tyr	Asp	Ile	Pro	Asp	Ser	Val	
				900						905				910			
	Ile	Ala	Phe	Leu	Arg	Gly	Glu	Leu	Gly	Thr	Pro	Pro	Gly	Gly	Trp	Pro	
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50	Glu	Pro	Leu	Arg	Thr	Arg	Ala	Leu	Glu	Gly	Arg	Ser	Gln	Gly	Lys	Ala	
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	Pro	Leu	Ala	Glu	Ile	Pro	Ala	Glu	Glu	Gln	Ala	His	Leu	Asp	Ser	Asp	
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 5 980 985 990
 Ala Leu Asp Asp Arg Glu Phe Phe Tyr Gly Leu Lys Glu Gly Arg Glu
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 Glu Leu Ile Arg Leu Thr Gly Val Ser Thr Pro Met Val Val Arg Leu
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 Asp Ala Val Ser Glu Pro Asp Asp Lys Gly Met Arg Asn Val Val Val
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 Val Glu Arg Ala Arg Arg Thr Ser Phe Asp Ile Ala Lys Gly Arg Ala

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	Glu Met Asp Ser Leu Val Glu Val Phe Ala Gly Ile Asp Pro Glu Asp			
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	gcc acg ccc glg gcc cga gcc ttc acc cat ttc gcc ctg ttg gcc aac			300
	Ala Thr Pro Val Ala Arg Ala Phe Thr His Phe Ala Leu Leu Ala Asn			
10	65	70	75	
	ctc gcg gag gat ttg cat gac gca gcc cag cgg gaa cag gcc ctg aac			348
	Leu Ala Glu Asp Leu His Asp Ala Ala Gln Arg Glu Gln Ala Leu Asn			
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	tcg ggt gag ccc gcg ccg gac agc acc ctc gag gcc acc tgg gtg aaa			396
	Ser Gly Glu Pro Ala Pro Asp Ser Thr Leu Glu Ala Thr Trp Val Lys			
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	ctg gat gat gcc ggg glg ggc agc ggt gag gtc gcc gcg gtg atc cgc			444
	Leu Asp Asp Ala Gly Val Gly Ser Gly Glu Val Ala Ala Val Ile Arg			
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	aat gcg ctc gtc gcc ccg glg ctc acc gcg cac ccg acg gaa acc cga			492
	Asn Ala Leu Val Ala Pro Val Leu Thr Ala His Pro Thr Glu Thr Arg			
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	cgt cgt acc glg ttc gac gcg cag aag cac atc acc gcc ctg atg gag			540
	Arg Arg Thr Val Phe Asp Ala Gln Lys His Ile Thr Ala Leu Met Glu			
35	145	150	155	
	gaa cgc cac ctc ctc ctg gcg ctg ccc acc cat gcc cgg acc cag tcc			588
	Glu Arg His Leu Leu Leu Ala Leu Pro Thr His Ala Arg Thr Gln Ser			
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	aag ctg gat gac atc gag cgc aac atc cgg cga cgg atc acg atc ctg			636
	Lys Leu Asp Asp Ile Glu Arg Asn Ile Arg Arg Arg Ile Thr Ile Leu			
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	tgg cag acg gcc ctc atc cgt glg gcc cgt ccc cgc atc gag gat gag			684
	Trp Gln Thr Ala Leu Ile Arg Val Ala Arg Pro Arg Ile Glu Asp Glu			
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	gtc gag gtt gga ctg cgc tac tac aag ctc agc ctg ttg gcc gag atc			732
	Val Glu Val Gly Leu Arg Tyr Tyr Lys Leu Ser Leu Leu Ala Glu Ile			
55	210	215	220	
	ccc cgc atc aat cat gat glg acc glg gaa ctg gcc cgg cgt ttc ggc			780
	Pro Arg Ile Asn His Asp Val Thr Val Glu Leu Ala Arg Arg Phe Gly			
60	225	230	235	
	ggg gat atc ccc acc acg gcg atg gtc agg ccg gga tcc tgg atc ggc			828
	Gly Asp Ile Pro Thr Thr Ala Met Val Arg Pro Gly Ser Trp Ile Gly			
65	240	245	250	255
	ggg gac cat gat ggc aac ccc ttc gtc acc gcg gag act gtc acc tac			876
	Gly Asp His Asp Gly Asn Pro Phe Val Thr Ala Glu Thr Val Thr Tyr			
70	260	265	270	

EP 1 219 712 A1

gcc acc cat cgg gcc gcg gag acc gtc ctc aag tac tac gtc aag caa 924
Ala Thr His Arg Ala Ala Glu Thr Val Leu Lys Tyr Tyr Val Lys Gln
275 280 285

5 ctc cac gcc ctc gaa cac gaa ctc agt ctc tcc gac cgg atg aac gtc 972
Leu His Ala Leu Glu His Glu Leu Ser Leu Ser Asp Arg Met Asn Val
290 295 300

10 atc agc gat gag ctc cgt gtc ctt gcc gat gcc gcc cag aat gac atg 1020
Ile Ser Asp Glu Leu Arg Val Leu Ala Asp Ala Gly Gln Asn Asp Met
305 310 315

15 ccc agc cgg gtt gat gaa ccc tac cgg cgg gcc atc cac gcc atg cgt 1068
Pro Ser Arg Val Asp Glu Pro Tyr Arg Arg Ala Ile His Gly Met Arg
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Gly Arg Met Leu Ala Thr Thr Ala Ala Leu Ile Gly Glu Glu Ala Val
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Glu Gly Thr Trp Phe Lys Thr Phe Thr Pro Tyr Thr Asp Thr His Glu
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25 ttc aaa cgc gac ctc gat atc gtc gat ggt tcc ctc aga atg tcc cgg 1212
Phe Lys Arg Asp Leu Asp Ile Val Asp Gly Ser Leu Arg Met Ser Arg
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gat gac atc atc gcc gat gac cgt ctc gcc atg ctc cgc tgc gcc ctc 1260
Asp Asp Ile Ile Ala Asp Asp Arg Leu Ala Met Leu Arg Ser Ala Leu
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30 gac agc ttc ggg ttc aac ctc tac tcc ctc gat ctc cgc cag aat tcc 1308
Asp Ser Phe Gly Phe Asn Leu Tyr Ser Leu Asp Leu Arg Gln Asn Ser
400 405 410 415

35 gac ggt ttc gag gat gtc ctc acc gaa ttc ttc gcc acc gcc cag acc 1356
Asp Gly Phe Glu Asp Val Leu Thr Glu Leu Phe Ala Thr Ala Gln Thr
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40 gag aag aac tac cgc ggg ttc acg gag gcc gag aag ctc gac ctc ctc 1404
Glu Lys Asn Tyr Arg Gly Leu Thr Glu Ala Glu Lys Leu Asp Leu Leu
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atc cgc gaa ctc agc aca ccc cgc ccg ctc atc ccg cac ggg gac ccg 1452
Ile Arg Glu Leu Ser Thr Pro Arg Pro Leu Ile Pro His Gly Asp Pro
450 455 460

45 gac tac tcc gag gcc acc aac cgt gaa ctc ggg att ttt tgc aag gcc 1500
Asp Tyr Ser Glu Ala Thr Asn Arg Glu Leu Gly Ile Phe Ser Lys Ala
465 470 475

50 gcg gag gcc gtc cgt aaa ttc ggt cct ctc atg gtc ccg cac tgc atc 1548
Ala Glu Ala Val Arg Lys Phe Gly Pro Leu Met Val Pro His Cys Ile
480 485 490 495

55 atc tcc atg gcc tct tcc gtc acg gac atc ctc gaa ccg atg gtc ctc 1596

	Ile Ser Met Ala Ser Ser Val Thr Asp Ile Leu Glu Pro Met Val Leu	
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5	ctc aag gag ttc ggt ctg atc cgg gcc aac ggg aag aac ccg acg ggc Leu Lys Glu Phe Gly Leu Ile Arg Ala Asn Gly Lys Asn Pro Thr Gly	1644
	515 520 525	
10	agc gtc gac gtg atc ccg ctg ttc gag acg atc gat gac ctc cag cgt Ser Val Asp Val Ile Pro Leu Phe Glu Thr Ile Asp Asp Leu Gln Arg	1692
	530 535 540	
15	ggc gcg ggc atc ctg gag gaa ttg tgg gac atc gac ctc tac cgc aat Gly Ala Gly Ile Leu Glu Glu Leu Trp Asp Ile Asp Leu Tyr Arg Asn	1740
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	580 585 590	
30	gac gcg gag tta cgc ctg gtc gaa cta tgc cgg ggc cgt aat gtc aag Asp Ala Glu Leu Arg Leu Val Glu Leu Cys Arg Gly Arg Asn Val Lys	1884
	595 600 605	
35	ctc cgt ctc ttc cac ggt cgt ggt ggc acg gtg ggt cgt ggc ggt ggc Leu Arg Leu Phe His Gly Arg Gly Gly Thr Val Gly Arg Gly Gly Gly	1932
	610 615 620	
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	625 630 635	
45	gcg gtg cgg gtg act gaa cag ggc gag atc atc tcc gcg aag tac ggt Ala Val Arg Val Thr Glu Gln Gly Glu Ile Ile Ser Ala Lys Tyr Gly	2028
	640 645 650 655	
50	aac ccg gat acg gca cgc cgc aac ctt gag gcc ctg gtg tcc gcg acg Asn Pro Asp Thr Ala Arg Arg Asn Leu Glu Ala Leu Val Ser Ala Thr	2076
	660 665 670	
55	ctg gag gca tgc ctt ctg gat gat gtg gaa ctg ccc aat cgg gaa cgc Leu Glu Ala Ser Leu Leu Asp Asp Val Glu Leu Pro Asn Arg Glu Arg	2124
	675 680 685	
60	gcg cac cag atc atg ggg gag atc tgc gag ttg agc ttc cgc agg tac Ala His Gln Ile Met Gly Glu Ile Ser Glu Leu Ser Phe Arg Arg Tyr	2172
	690 695 700	
65	tca tca ctg gtc cat gag gat ccc gga ttc atc cag tac ttc acc cag Ser Ser Leu Val His Glu Asp Pro Gly Phe Ile Gln Tyr Phe Thr Gln	2220
	705 710 715	
70	tcc acc ccc ctg cag gag atc gga tcc ctc aac atc ggt tcc cga ccc Ser Thr Pro Leu Gln Glu Ile Gly Ser Leu Asn Ile Gly Ser Arg Pro	2268

720 725 730 735
 tcc tca cgt aaa cag acc aac acg glg gag gal clg cgl gcc alc ccg 2316
 Ser Ser Arg Lys Gln Thr Asn Thr Val Glu Asp Leu Arg Ala Ile Pro
 5 740 745 750
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 ggt glg ggt acc gca clg cgt gag tgg atc ggt gag ggg gag ggg gct 2412
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 15 770 775 780
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 Ala Glu Arg Ile Ala Glu Leu Gln Glu Leu Asn Arg Cys Trp Pro Phe
 20 785 790 795
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 Phe Thr Ser Val Leu Asp Asn Met Ala Gln Val Met Ser Lys Ala Glu
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 Pro Ala Leu Ala Arg Ser Val Arg Ser Arg Phe Pro Tyr Leu Leu Pro
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Ala Glu Asp Leu His Asp Ala Ala Gln Arg Glu Gln Ala Leu Asn Ser

85 90 95

Gly Glu Pro Ala Pro Asp Ser Thr Leu Glu Ala Thr Trp Val Lys Leu

100 105 110

Asp Asp Ala Gly Val Gly Ser Gly Glu Val Ala Ala Val Ile Arg Asn

115 120 125

Ala Leu Val Ala Pro Val Leu Thr Ala His Pro Thr Glu Thr Arg Arg

130 135 140

Arg Thr Val Phe Asp Ala Gln Lys His Ile Thr Ala Leu Met Glu Glu

145 150 155 160

Arg His Leu Leu Leu Ala Leu Pro Thr His Ala Arg Thr Gln Ser Lys

165 170 175

Leu Asp Asp Ile Glu Arg Asn Ile Arg Arg Arg Ile Thr Ile Leu Trp

180 185 190

Gln Thr Ala Leu Ile Arg Val Ala Arg Pro Arg Ile Glu Asp Glu Val

195 200 205

Glu Val Gly Leu Arg Tyr Tyr Lys Leu Ser Leu Leu Ala Glu Ile Pro

210 215 220

Arg Ile Asn His Asp Val Thr Val Glu Leu Ala Arg Arg Phe Gly Gly

225 230 235 240

Asp Ile Pro Thr Thr Ala Met Val Arg Pro Gly Ser Trp Ile Gly Gly

245 250 255

Asp His Asp Gly Asn Pro Phe Val Thr Ala Glu Thr Val Thr Tyr Ala

260 265 270

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 305 310 315 320
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 Lys Arg Asp Leu Asp Ile Val Asp Gly Ser Leu Arg Met Ser Arg Asp
 370 375 380
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 Ser Phe Gly Phe Asn Leu Tyr Ser Leu Asp Leu Arg Gln Asn Ser Asp
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 25 Gly Phe Glu Asp Val Leu Thr Glu Leu Phe Ala Thr Ala Gln Thr Glu
 420 425 430
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 435 440 445
 30 Arg Glu Leu Ser Thr Pro Arg Pro Leu Ile Pro His Gly Asp Pro Asp
 450 455 460
 Tyr Ser Glu Ala Thr Asn Arg Glu Leu Gly Ile Phe Ser Lys Ala Ala
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 45 Ala Gly Ile Leu Glu Glu Leu Trp Asp Ile Asp Leu Tyr Arg Asn Tyr
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	Pro Asp Thr Ala Arg Arg Asn Leu	Glu Ala Leu Val Ser Ala Thr Leu				
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	Glu Ala Ser Leu Leu Asp Asp Val	Glu Leu Pro Asn Arg Glu Arg Ala				
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	Ser Leu Val His Glu Asp Pro Gly Phe Ile	Gln Tyr Phe Thr Gln Ser				
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	Thr Pro Leu Gln Glu Ile Gly Ser Leu Asn	Ile Gly Ser Arg Pro Ser				
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	Ser Arg Lys Gln Thr Asn Thr Val	Glu Asp Leu Arg Ala Ile Pro Trp				
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	Val Leu Ser Trp Ser Gln Ser Arg Val Met	Leu Pro Gly Trp Phe Gly				
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	Glu Arg Ile Ala Glu Leu Gln Glu Leu Asn	Arg Cys Trp Pro Phe Phe				
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	Arg Leu Ala Arg Leu Tyr Ala Asp Leu Ile	Pro Asp Arg Glu Val Ala				
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	Asp Arg Ile Tyr Glu Thr Ile Phe Gly Glu Tyr	Phe Leu Thr Lys Glu				
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	Ala Leu Ala Arg Ser Val Arg Ser Arg Phe	Pro Tyr Leu Leu Pro Leu				
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	Asn Val Ile Gln Val Glu Met Met Arg Arg	Tyr Arg Ser Gly Asp Glu				
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 tatagaaatt caagggggat atcaa atg gct tct aat ttt aaa gaa aca gcg 712
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 Ser Ile Arg Val Leu Leu Glu Ser Val Leu Arg Gln Glu Asp Asp Phe
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 gta att act gat gat cac att aaa caa tta gca gaa ttt ggc aaa aaa 904
 Val Ile Thr Asp Asp His Ile Lys Gln Leu Ala Glu Phe Gly Lys Lys
 60 65 70
 ggt aac gaa ggt gaa gta cct ttc aaa cca tct cga gtt att tta caa 952
 Gly Asn Glu Gly Glu Val Pro Phe Lys Pro Ser Arg Val Ile Leu Gln
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 Asp Phe Thr Gly Val Pro Ala Val Val Asp Leu Ala Ser Leu Arg Lys
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 gca atg aat gat gtt ggt ggg gat att aat aaa att aac cct gaa gta 1048
 Ala Met Asn Asp Val Gly Gly Asp Ile Asn Lys Ile Asn Pro Glu Val
 110 115 120
 cca gtt gac tta gtt att gac cac tct gta caa gla gat agt tat gct 1096

Pro Val Asp Leu Val Ile Asp His Ser Val Gln Val Asp Ser Tyr Ala

125

130

135

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	Glu Tyr Leu Ala Asn Val Val His Val Arg Asp Val Asp Gly Glu Gln	
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	Thr Ala Phe Pro Asp Thr Leu Val Gly Thr Asp Ser His Thr Thr Met	
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	Ile Asn Gly Ile Gly Val Leu Gly Trp Gly Val Gly Gly Ile Glu Ala	
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	Glu Ala Gly Met Leu Gly Gln Pro Ser Tyr Phe Pro Ile Pro Glu Val	
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	Gly Lys Phe Val Glu Phe Phe Gly Pro Gly Val Thr Asn Leu Pro Leu	
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	Cys Gly Phe Phe Pro Val Asp Glu Glu Ser Leu Lys Tyr Met Lys Leu	
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	Thr Gly Arg Lys Asp Asp His Ile Ala Leu Val Lys Glu Tyr Leu Gln	
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	Gln Asn Asn Met Phe Phe Gln Val Glu Asn Glu Asp Pro Glu Tyr Thr	

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	Arg Thr Ser Thr Met Lys Thr Gly Asp Val Ala Ile Ala Ala Ile Thr			
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55	Gly Thr Val Asp Ile Asp Leu His Asn Glu Pro Ile Gly Lys Gly Lys			
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 5 aaa caa ggt gag tca gct gat tct cta ggt tta gaa ggt aaa gaa gaa 3208
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 Ile Ser Val Asp Ile Asp Glu Asn Val Lys Pro His Asp Leu Val Thr
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	Thr	Ser	Gly	Lys	Met	Trp	Asn	Lys	Asp	Asp	Gln	Thr	Gln	Asp	Ala	Leu	
					350						355				360		
30	gct	gtc	atc	ccg	gac	tcc	tcc	tac	gcc	ggt	gtc	tac	cag	acc	gtc	atc	1458
	Ala	Val	Ile	Pro	Asp	Ser	Ser	Tyr	Ala	Gly	Val	Tyr	Gln	Thr	Val	Ile	
				365					370					375			
35	gag	gac	tgc	cgc	aag	aal	ggc	gcc	ttc	gat	ccg	acc	acc	atg	ggc	acc	1506
	Glu	Asp	Cys	Arg	Lys	Asn	Gly	Ala	Phe	Asp	Pro	Thr	Thr	Met	Gly	Thr	
				380				385						390			
40	gtc	ccc	aac	gtc	ggt	ctg	atg	gca	cag	aag	gcc	gag	gag	tac	ggc	tcc	1554
	Val	Pro	Asn	Val	Gly	Leu	Met	Ala	Gln	Lys	Ala	Glu	Glu	Tyr	Gly	Ser	
				395				400						405			
45	cac	gac	aag	acc	ttc	cgt	atc	gag	gcc	gac	ggc	aag	gta	cag	gtc	gtc	1602
	His	Asp	Lys	Thr	Phe	Arg	Ile	Glu	Ala	Asp	Gly	Lys	Val	Gln	Val	Val	
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	Ala	Ser	Asn	Gly	Asp	Val	Leu	Ile	Glu	His	Asp	Val	Glu	Lys	Gly	Asp	
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	Ile	Trp	Arg	Ala	Cys	Gln	Thr	Lys	Asp	Ala	Pro	Ile	Gln	Asp	Trp	Val	
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60	aag	ctg	gct	gtc	aac	cgc	gca	cgt	ctc	tcc	ggc	atg	ccc	gct	gtg	ttc	1746
	Lys	Leu	Ala	Val	Asn	Arg	Ala	Arg	Leu	Ser	Gly	Met	Pro	Ala	Val	Phe	
				460				465						470			
65	tgg	ctg	gat	ccc	gcc	cgc	gca	cac	gac	cgc	aac	ctg	acc	aca	ctg	gtg	1794
	Trp	Leu	Asp	Pro	Ala	Arg	Ala	His	Asp	Arg	Asn	Leu	Thr	Thr	Leu	Val	
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	Glu	Lys	Tyr	Leu	Ala	Asp	His	Asp	Thr	Glu	Gly	Leu	Asp	Ile	Gln	Ile	

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	ggc gag gac acc atc tcc gtc acc ggt aac gtc ctg cgt gac tac aac	1938						
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	acc gac ctc ttc ccg atc ctc gag ctg ggc acc tcc gcc aag atg ctc	1986						
	Thr Asp Leu Phe	Pro Ile Leu Glu Leu Gly Thr Ser Ala Lys Met Leu						
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	ggt ggc tcc gcc ccg aag cac gtc cag cag gtc atc gag gaa aac cac	2082						
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	Leu Arg Trp Asp	Ser Leu Gly Glu Phe Leu Ala Leu Ala Glu Ser Phe						
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	cgc cac gag ctc aac acc cgc aac aac acc aag gcc ggt gtc ctc gcc	2178						
	Arg His Glu Leu	Asn Thr Arg Asn Asn Thr Lys Ala Gly Val Leu Ala						
		605 610 615						
30	gat gcc ctg gac cgt gcg acc gag aag ctc ctc aac gag gag aag tcc	2226						
	Asp Ala Leu Asp	Arg Ala Thr Glu Lys Leu Leu Asn Glu Glu Lys Ser						
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	ccg tcc cgc aag gtc ggc gag atc gac aac cgt ggt tcc cac ttc tgg	2274						
35	Pro Ser Arg Lys	Val Gly Glu Ile Asp Asn Arg Gly Ser His Phe Trp						
		635 640 645						
	ctg gcc acc tac tgg gcc gat gaa ctg gcc aac cag acc gag gac gcc	2322						
	Leu Ala Thr Tyr	Trp Ala Asp Glu Leu Ala Asn Gln Thr Glu Asp Ala						
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	gag ctg gct gag acc ttc gcc cct gtc gcc gag gcc ctg aac aac cag	2370						
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	Ala Ala Asp Ile	Asp Ala Ala Leu Ile Gly Glu Gln Gly Lys Pro Val						
		685 690 695						
	gac ctg ggt ggc tac tac gca ccc tcc gat gag aag acc tcc gcg atc	2466						
50	Asp Leu Gly Gly	Tyr Tyr Ala Pro Ser Asp Glu Lys Thr Ser Ala Ile						
		700 705 710						
	atg cgc ccg glg gcc gca ttc aac gag atc atc gac tcc ctg aag aag	2514						
55	Met Arg Pro Val	Ala Ala Phe Asn Glu Ile Ile Asp Ser Leu Lys Lys						
		715 720 725						

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<213> *Corynebacterium thermoaminogenes*

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20 25 30

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35 40 45

Gln Phe Ala Asp Gln Leu Pro Glu Glu Gln Lys Val Ser Asp Ala Leu

50 55 60

Ala Glu Leu Gly Glu Leu Ala Lys Thr Pro Glu Ala Asn Ile Ile Lys

65 70 75 80

Leu Pro Asn Ile Ser Ala Ser Val Pro Gln Leu Lys Ala Ala Val Lys

85 90 95

Glu Leu Gln Glu Gln Gly Tyr Asp Leu Pro Glu Tyr Glu Asp Ala Lys

100 105 110

Asp Arg Tyr Ala Ala Val Ile Gly Ser Asn Val Asn Pro Val Leu Arg

115 120 125

Glu Gly Asn Ser Asp Arg Arg Ala Pro Val Ala Val Lys Asn Phe Val

130 135 140

Lys Lys Phe Pro His Arg Met Gly Glu Trp Ser Ala Asp Ser Lys Thr

145 150 155 160

Asn Val Ala Thr Met Gly Ala Asp Asp Phe Arg Ser Asn Glu Lys Ser

165 170 175

Val Ile Met Asp Glu Ala Asp Thr Val Val Ile Lys His Val Ala Ala

180 185 190

Asp Gly Thr Glu Thr Val Leu Lys Asp Ser Leu Pro Leu Leu Lys Gly

195 200 205

Glu Val Ile Asp Gly Thr Phe Ile Ser Ala Lys Ala Leu Asp Ala Phe

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	225					230					235					240
5	Ala	His	Met	Lys	Ala	Thr	Met	Met	Lys	Val	Ser	Asp	Pro	Ile	Ile	Phe
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	Gly	His	Ile	Val	Arg	Ala	Tyr	Phe	Ala	Asp	Val	Tyr	Ala	Gln	Tyr	Gly
				260						265					270	
10	Glu	Gln	Leu	Leu	Ala	Ala	Gly	Leu	Asn	Gly	Glu	Asn	Gly	Leu	Ala	Ala
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	Ile	Tyr	Ala	Gly	Leu	Asp	Lys	Leu	Asp	Asn	Gly	Ala	Glu	Ile	Lys	Ala
				290						295					300	
15	Ala	Phe	Asp	Lys	Gly	Leu	Glu	Glu	Gly	Pro	Asp	Leu	Ala	Met	Val	Asn
	305					310					315					320
	Ser	Ala	Lys	Gly	Ile	Thr	Asn	Leu	His	Val	Pro	Ser	Asp	Val	Ile	Ile
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	Lys	Asp	Asp	Gln	Thr	Gln	Asp	Ala	Leu	Ala	Val	Ile	Pro	Asp	Ser	Ser
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	385						390				395					400
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35	Ile	Glu	His	Asp	Val	Glu	Lys	Gly	Asp	Ile	Trp	Arg	Ala	Cys	Gln	Thr
				435						440					445	
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				450						455					460	
40	Arg	Leu	Ser	Gly	Met	Pro	Ala	Val	Phe	Trp	Leu	Asp	Pro	Ala	Arg	Ala
	465						470				475					480
	His	Asp	Arg	Asn	Leu	Thr	Thr	Leu	Val	Glu	Lys	Tyr	Leu	Ala	Asp	His
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50	Thr	Gly	Asn	Val	Leu	Arg	Asp	Tyr	Asn	Thr	Asp	Leu	Phe	Pro	Ile	Leu
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	Glu	Leu	Gly	Thr	Ser	Ala	Lys	Met	Leu	Ser	Val	Pro	Leu	Met	Ala	
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565

570

575

Val Gln Gln Val Ile Glu Glu Asn His Leu Arg Trp Asp Ser Leu Gly

580

585

590

Glu Phe Leu Ala Leu Ala Glu Ser Phe Arg His Glu Leu Asn Thr Arg

595

600

605

Asn Asn Thr Lys Ala Gly Val Leu Ala Asp Ala Leu Asp Arg Ala Thr

610

615

620

Glu Lys Leu Leu Asn Glu Glu Lys Ser Pro Ser Arg Lys Val Gly Glu

625

630

635

640

Ile Asp Asn Arg Gly Ser His Phe Trp Leu Ala Thr Tyr Trp Ala Asp

645

650

655

Glu Leu Ala Asn Gln Thr Glu Asp Ala Glu Leu Ala Glu Thr Phe Ala

660

665

670

Pro Val Ala Glu Ala Leu Asn Asn Gln Ala Ala Asp Ile Asp Ala Ala

675

680

685

Leu Ile Gly Glu Gln Gly Lys Pro Val Asp Leu Gly Gly Tyr Tyr Ala

690

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ccitagccctc gccgaigcta aaagtcagct gaccccttgg ggccgttcat ttgaaactgc 180

gaccaagctc atgaatgcgc gaaagcattt ccattataag ggtaagcigt aagaatagtg 240

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atgallatgt attgtataag cctcaaagac cgaalagatt actaacattt aagtggaacca 420

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 Asp Cys Ile Ile Ala Thr Gly Ser Val Val Asn Ser Leu Arg Gly Val
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 45 Glu Phe Ser Glu Asn Val Val Ser Tyr Glu Glu Gln Ile Leu Asn Pro
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 Val Ala Pro Lys Lys Met Val Ile Val Gly Gly Gly Ala Ile Gly Met
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 55 gag ttc atg gac cgc gtt ctg ccg aac gag gat cca gag gtg tcc aag 1456

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	Glu	Phe	Met	Asp	Arg	Val	Leu	Pro	Asn	Glu	Asp	Pro	Glu	Val	Ser	Lys	
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5	gtt	atc	gcc	aag	gcc	tac	aag	aag	atg	ggc	atc	aag	cic	cic	ccg	ggc	1504
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10	cac	gca	acc	acc	gcg	gig	cgc	gac	aal	ggc	gal	icc	glt	gag	gic	gal	1552
	His	Ala	Thr	Thr	Ala	Val	Arg	Asp	Asn	Gly	Asp	Ser	Val	Glu	Val	Asp	
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	Tyr	Gln	Lys	Lys	Gly	Ser	Asp	Lys	Thr	Glu	Thr	Ile	Thr	Val	Asp	Arg	
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	Val	Leu	Ile	Ser	Val	Gly	Phe	Arg	Pro	Arg	Val	Glu	Gly	Phe	Gly	Leu	
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	Glu	Asn	Thr	Gly	Val	Lys	Leu	Thr	Glu	Arg	Gly	Ala	Ile	Asp	Ile	Asp	
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	Glu	His	Met	Arg	Thr	Asn	Val	Asp	Gly	Ile	Tyr	Ala	Ile	Gly	Asp	Val	
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	Thr	Ala	Lys	Leu	Gln	Leu	Ala	His	Val	Ala	Glu	Ala	Gln	Gly	Ile	Val	
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	Met	Met	Met	Pro	Arg	Ala	Thr	Phe	Cys	Asn	Pro	Gln	Val	Ala	Ser	Phe	
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	Gly	Tyr	Thr	Glu	Glu	Gln	Ala	Lys	Glu	Lys	Trp	Pro	Asp	Arg	Glu	Ile	
				365						370					375		
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	Ala	Glu	Thr	Asp	Gly	Phe	Ala	Lys	Ile	Val	Ala	Asp	Ala	Glu	Phe	Gly	
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	Glu	Leu	Leu	Gly	Gly	His	Ile	Val	Gly	Ala	Asn	Ala	Ser	Glu	Leu	Leu	
	410						415					420				425	
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	Asn	Glu	Leu	Val	Leu	Ala	Gln	Asn	Trp	Asp	Leu	Thr	Thr	Glu	Glu	Ile	

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 Ser Arg Ser Val His Ile His Pro Thr Leu Ser Glu Ala Val Lys Glu
 5 445 450 455
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 35 40 45
 Pro Ser Lys Ala Leu Ile Lys Asn Ala Glu Ile Ala His Ile Phe Asn
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 His Glu Lys Lys Thr Phe Gly Ile Asn Gly Glu Val Thr Phe Asn Tyr
 65 70 75 80
 Glu Asp Ala His Lys Arg Ser Arg Gly Val Ser Asp Lys Ile Val Gly
 35 85 90 95
 Gly Val His Tyr Leu Met Lys Lys Asn Lys Ile Thr Glu Ile Asp Gly
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 Phe Gly Thr Phe Lys Asp Ala Lys Thr Ile Glu Val Thr Asp Gly Lys
 40 115 120 125
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 Ser Tyr Glu Glu Gln Ile Leu Asn Pro Val Ala Pro Lys Lys Met Val
 165 170 175
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 Asn Tyr Gly Val Asp Val Thr Leu Ile Glu Phe Met Asp Arg Val Leu
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 55 Pro Asn Glu Asp Pro Glu Val Ser Lys Val Ile Ala Lys Ala Tyr Lys

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	Lys Thr Glu Thr Ile Thr Val Asp Arg Val Leu Ile Ser Val Gly Phe		
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	Arg Pro Arg Val Glu Gly Phe Gly Leu Glu Asn Thr Gly Val Lys Leu		
	275	280	285
	Thr Glu Arg Gly Ala Ile Asp Ile Asp Glu His Met Arg Thr Asn Val		
15	290	295	300
	Asp Gly Ile Tyr Ala Ile Gly Asp Val Thr Ala Lys Leu Gln Leu Ala		
	305	310	315 320
	His Val Ala Glu Ala Gln Gly Ile Val Ala Ala Glu Thr Leu Ala Gly		
20	325	330	335
	Ala Glu Thr Gln Thr Leu Gly Asp Tyr Met Met Met Pro Arg Ala Thr		
	340	345	350
	Phe Cys Asn Pro Gln Val Ala Ser Phe Gly Tyr Thr Glu Glu Gln Ala		
25	355	360	365
	Lys Glu Lys Trp Pro Asp Arg Glu Ile Lys Val Ser Ser Phe Pro Phe		
	370	375	380
	Ser Ala Asn Gly Lys Ala Val Gly Leu Ala Glu Thr Asp Gly Phe Ala		
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	Lys Ile Val Ala Asp Ala Glu Phe Gly Glu Leu Leu Gly Gly His Ile		
	405	410	415
	Val Gly Ala Asn Ala Ser Glu Leu Leu Asn Glu Leu Val Leu Ala Gln		
35	420	425	430
	Asn Trp Asp Leu Thr Thr Glu Glu Ile Ser Arg Ser Val His Ile His		
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 Ala Ser Lys Pro Ala Lys Lys Ala Lys Glu Ser Pro Leu Ser Lys Pro
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 His Ile Ile Gly Tyr Ala Met Val Lys Ala Val Met Ala His Pro Asp
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	Trp Asp Leu Asp Arg Thr Phe His Val Gly Gly Phe Gly Gly Lys Glu	
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	Gln Asp Ile Leu Asp Lys Gly Pro Asp Gly Tyr Thr Val Val Pro Leu	
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	Glu Leu Ala Gln Ala Lys Gly Gly Gly Lys Phe Leu Val Tyr Asn Ser			
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	Gly Asn Pro Asp Ala Val Val Ser Trp Glu Ala Gln Phe Gly Asp Phe			
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	Pro Ala Asn Tyr Phe His Leu Leu Arg Arg His Ala Leu Gly Lys Met			
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 Glu Lys Asp Asn Arg Asp Asp Ile Ala Ile Val Arg Ile Glu Met Leu

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Ser Gly Gln Ser Thr Ala Lys Ala Ala Pro Ala Ala Lys Thr Ala Pro

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	Gly	Gln	Gly	Thr																												

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	610						615					620				
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	625					630					635					640
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 805 810 815
 Val Phe Asn Glu Val Lys Glu Ala Gly Lys Lys Gln Pro Asp Glu Gln
 820 825 830
 Thr Gly Ile Thr Gly Ser Gln Glu Leu Thr Arg Gly Leu Asp Thr Asn
 835 840 845
 Ile Thr Arg Glu Glu Leu Val Glu Leu Gly Gln Ala Phe Val Asn Thr
 850 855 860
 Pro Glu Gly Phe Thr Tyr His Pro Arg Val Ala Pro Val Ala Lys Lys
 865 870 875 880
 Arg Ala Glu Ser Val Thr Glu Gly Gly Ile Asp Trp Ala Trp Gly Glu
 885 890 895
 Leu Ile Ala Phe Gly Ser Leu Ala Thr Ser Gly Arg Leu Val Arg Leu
 900 905 910
 Ala Gly Glu Asp Ser Arg Arg Gly Thr Phe Thr Gln Arg His Ala Val
 915 920 925
 Ala Ile Asp Pro Asn Thr Ala Glu Glu Phe Asn Pro Leu His Glu Leu
 930 935 940
 Ala Gln Ala Lys Gly Gly Gly Lys Phe Leu Val Tyr Asn Ser Ala Leu
 945 950 955 960
 Thr Glu Tyr Ala Gly Met Gly Phe Glu Tyr Gly Tyr Ser Val Gly Asn
 965 970 975
 Pro Asp Ala Val Val Ser Trp Glu Ala Gln Phe Gly Asp Phe Ala Asn
 980 985 990
 Gly Ala Gln Thr Ile Ile Asp Glu Tyr Ile Ser Ser Gly Glu Ala Lys
 995 1000 1005
 Trp Gly Gln Thr Ser Ser Val Ile Leu Leu Leu Pro His Gly Tyr Glu
 1010 1015 1020
 Gly Gln Gly Pro Asp His Ser Ser Ala Arg Ile Glu Arg Phe Leu Gln
 1025 1030 1035 1040
 Leu Cys Ala Glu Gly Ser Met Thr Ile Ala Gln Pro Thr Thr Pro Ala
 1045 1050 1055
 Asn Tyr Phe His Leu Leu Arg Arg His Ala Leu Gly Lys Met Lys Arg
 1060 1065 1070
 Pro Leu Val Val Phe Thr Pro Lys Ser Met Leu Arg Asn Lys Ala Ala
 1075 1080 1085
 Thr Ser Ala Pro Glu Glu Phe Thr Glu Val Thr Arg Phe Lys Ser Val
 1090 1095 1100
 Ile Asp Asp Pro Asn Val Ala Asp Ala Ser Lys Val Lys Lys Ile Met
 105 110 1115 1120

Leu Cys Ser Gly Lys Ile Tyr Tyr Glu Leu Ala Lys Arg Lys Glu Lys
 1125 1130 1135

5 Asp Asn Arg Asp Asp Ile Ala Ile Val Arg Ile Glu Met Leu His Pro
 1140 1145 1150
 Ile Pro Phe Asn Arg Leu Arg Asp Ala Phe Asp Gly Tyr Pro Asn Ala
 1155 1160 1165
 10 Glu Glu Ile Leu Phe Val Gln Asp Glu Pro Ala Asn Gln Gly Ala Trp
 1170 1175 1180
 Pro Phe Tyr Gln Glu His Leu Pro Asn Leu Ile Glu Gly Met Leu Pro
 185 1190 1195 1200
 15 Met Arg Arg Ile Ser Arg Arg Ser Gln Ser Ser Thr Ala Thr Gly Ile
 1205 1210 1215
 Ala Lys Val His Thr Ile Glu Gln Gln Lys Leu Leu Asp Asp Ala Phe
 1220 1225 1230
 20 Asn Ala

<210> 35
 <211> 20
 25 <212> DNA
 <213> Artificial Sequence

<220>
 30 <223> Description of Artificial Sequence: primer for aceA

<400> 35
 35 ccctctaccca gcgaactccg

20

<210> 36
 <211> 20
 40 <212> DNA
 <213> Artificial Sequence

<220>
 45 <223> Description of Artificial Sequence: primer for aceA

<400> 36
 50 ctgcccttgaa ctacagggttc

20

<210> 37
 <211> 20
 55 <212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer for accBC

5 <400> 37

catccacccc ggclacggct

20

10 <210> 38

<211> 20

<212> DNA

<213> Artificial Sequence

15 <220>

<223> Description of Artificial Sequence: primer for accBC

20 <400> 38

cggtagcagg gtagtccacc

20

<210> 39

25 <211> 20

<212> DNA

<213> Artificial Sequence

30 <220>

<223> Description of Artificial Sequence: primer for disR1

<400> 39

35 acgccccagc cctgaccgac

20

<210> 40

<211> 20

40 <212> DNA

<213> Artificial Sequence

<220>

45 <223> Description of Artificial Sequence: primer for disR1

<400> 40

50 agcagcgccc atgacggcga

20

<210> 41

<211> 20

55 <212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer for dtsR2

5

<400> 41

acggcccagc cctgaccgac

20

10

<210> 42

<211> 20

<212> DNA

<213> Artificial Sequence

15

<220>

<223> Description of Artificial Sequence: primer for dtsR2

20

<400> 42

agcagcgccc atgacggcga

20

25

<210> 43

<211> 20

<212> DNA

<213> Artificial Sequence

30

<220>

<223> Description of Artificial Sequence: primer for pfk

35

<400> 43

cgtaatccga ggaatcgtcc

20

40

<210> 44

<211> 20

<212> DNA

<213> Artificial Sequence

45

<220>

<223> Description of Artificial Sequence: primer for pfk

50

<400> 44

cgtagcggcc catgacctcc

21

55

<210> 45

<211> 17

<212> DNA

5 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: primer for scrB
 <220>
 10 <221> UNSURE
 <222> (3)
 <223> n=a or g or c or t
 15 <400> 45
 ggncghytba aygaycc 17
 <210> 46
 20 <211> 20
 <212> DNA
 <213> Artificial Sequence
 25 <220>
 <223> Description of Artificial Sequence: primer for scrB
 <220>
 30 <221> UNSURE
 <222> (18)
 <223> n=a or g or c or t
 35 <400> 46
 ggrcaytccc acatrtance 20
 <210> 47
 40 <211> 20
 <212> DNA
 <213> Artificial Sequence
 45 <220>
 <223> Description of Artificial Sequence: primer for gluABCD
 <400> 47
 50 ccatccggat ccggcaagtc 20
 <210> 48
 55 <211> 20
 <212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer for gluABCD

<400> 48

aatcccatct cgtgggtaac

20

<210> 49

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer for pdhA

<400> 49

acigtgcca tgggtcttgg ccc

23

<210> 50

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer for pdhA

<400> 50

cgctggaatccgaacatcga

20

<210> 51

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer for pc

<400> 51

ggcgcaacct acgacgttgc aatgcg

26

<210> 52

<211> 20

<212> DNA
<213> Artificial Sequence

5 <220>
<223> Description of Artificial Sequence: primer for pc

10 <400> 52
tggccgctg ggaclcgta 20

<210> 53
15 <211> 20
<212> DNA
<213> Artificial Sequence

20 <220>
<223> Description of Artificial Sequence: primer for ppc

25 <400> 53
ggllccigga ttggtggaga 20

<210> 54
30 <211> 20
<212> DNA
<213> Artificial Sequence

35 <220>
<223> Description of Artificial Sequence: primer for ppc

40 <400> 54
ccgccatcct tgttggatc 20

<210> 55
45 <211> 20
<212> DNA
<213> Artificial Sequence

50 <220>
<223> Description of Artificial Sequence: primer for acn

<220>
<221> UNSURE
<222> (3,6,9)
55 <223> n=inosine

<400> 55

gtingnacng aylcscalac

20

<210> 56

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer for acn

<220>

<221> UNSURE

<222> (3, 9, 18)

<223> n=inosine

<400> 56

gcnggagana tgtgrlcngt

20

<210> 57

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer for icd

<400> 57

gacatlacac tgcctggacg

20

<210> 58

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer for icd

<400> 58

ccglactctt cagcccttcg

20

<210> 59

<211> 17
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: primer for lpd

<400> 59
 atcatcgcaa ccggttc

17

<210> 60
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: primer for lpd

<400> 60
 cgtcaccgat ggcgtaaat

19

<210> 61
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: primer for odhA

<400> 61
 acaccgtggc cgccacaacg

20

<210> 62
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: primer for odhA

<400> 62
 lgctaaccgc tcccacctgg

20

<210> 63

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer for
screening PCR of lpd

<400> 63

tacgaggagc agatcctcaa

20

<210> 64

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer for
screening PCR of lpd

<400> 64

ttagcgccgg tgtctccag

20

<210> 65

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer for
LA cloning of acn

<400> 65

ggtgaagcta agtagttagc

20

<210> 66

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer for

LA cloning of acn

5 <400> 66
agctactaaa cctgcacc 18

10 <210> 67
 <211> 20
 <212> DNA
 <213> Artificial Sequence

15 <220>
 <223> Description of Artificial Sequence: primer for
 LA cloning of icd

20 <400> 67
ccglactctt cagccttcg 67

25 <210> 68
 <211> 18
 <212> DNA
 <213> Artificial Sequence

30 <220>
 <223> Description of Artificial Sequence: primer for
 LA cloning of icd

35 <400> 68
tcglccttgt tccacatc 18

40 <210> 69
 <211> 17
 <212> DNA
 <213> Artificial Sequence

45 <220>
 <223> Description of Artificial Sequence: primer for
 LA cloning of lpd

50 <400> 69
atcatcgcaa ccggttc 17

55 <210> 70
 <211> 20

<212> DNA

<213> Artificial Sequence

5 <220>

<223> Description of Artificial Sequence: primer for
LA cloning of lpd

10 <400> 70

tacgaggagc agatcccaaa

20

15 <210> 71

<211> 20

<212> DNA

<213> Artificial Sequence

20 <220>

<223> Description of Artificial Sequence: primer for
LA cloning of acn

25 <400> 71

gctaaactact tagcttcacc

20

30 <210> 72

<211> 20

<212> DNA

<213> Artificial Sequence

35 <220>

<223> Description of Artificial Sequence: primer for
LA cloning of acn

40 <400> 72

gaaccaggaa ctattgaacc

20

45 <210> 73

<211> 18

<212> DNA

<213> Artificial Sequence

50 <220>

<223> Description of Artificial Sequence: primer for
LA cloning of icd

<400> 73

tccgatgtca tcatcgac

18

<210> 74

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer for
LA cloning of icd

<400> 74

atgtggaaca aggacgac

18

<210> 75

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer for
LA cloning of odhA

<400> 75

glacatatlg tcgttagaac gcgtaatacg acica

35

<210> 76

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer for
LA cloning of odhA

<400> 76

cglttagaacg cglaatacga ctactatag ggaga

35

<210> 77

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer for
amplifying gdh gene

<400> 77

gcgccctgcag gtccgagggt gtgcgttcgg ca

32

<210> 78

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer for
amplifying gdh gene

<400> 78

gcgccctgcag ccaccagga tgcctcaacc ag

32

<210> 79

<211> 1344

<212> DNA

<213> Corynebacterium thermoaminogenes

<220>

<221> CDS

<222> (1).. (1341)

<400> 79

atg act gta gat gag cag gtc tcc aac tac tac gac atg ctg ctg aag 48

Met Thr Val Asp Glu Gln Val Ser Asn Tyr Tyr Asp Met Leu Leu Lys

1 5 10 15

cgc aac gcc ggg gaa cct gag ttc cac cag gct gtc gcg gag gtt ctc 96

Arg Asn Ala Gly Glu Pro Glu Phe His Gln Ala Val Ala Glu Val Leu

20 25 30

gaa tct ctg aag atc gtc ctg gag aag gac ccg cac tac gcc gac tac 144

Glu Ser Leu Lys Ile Val Leu Glu Lys Asp Pro His Tyr Ala Asp Tyr

35 40 45

ggt ctg atc cag cgt ctc tgc gaa ccg gaa cgc cag ctg atc ttc cgt 192

	Gly Leu Ile Gln Arg Leu Cys Glu Pro Glu Arg Gln Leu Ile Phe Arg	
	50 55 60	
5	gtg ccc tgg gtg gat gac aac ggt cag gtg cac gtc aac cgt ggt ttc	240
	Val Pro Trp Val Asp Asp Asn Gly Gln Val His Val Asn Arg Gly Phe	
	65 70 75 80	
10	cgt gtc cag ttc aac tcc gca ctc ggc ccg tac aag ggt ggt ctg cgt	288
	Arg Val Gln Phe Asn Ser Ala Leu Gly Pro Tyr Lys Gly Gly Leu Arg	
	85 90 95	
15	ttc cac ccc tcc gtc aac ctc ggc atc gtc aag ttc ctc ggc ttc gag	336
	Phe His Pro Ser Val Asn Leu Gly Ile Val Lys Phe Leu Gly Phe Glu	
	100 105 110	
20	cag atc ttc aag aac tcc ctc acc ggt ctg ccg atc ggt ggc ggc aag	384
	Gln Ile Phe Lys Asn Ser Leu Thr Gly Leu Pro Ile Gly Gly Gly Lys	
	115 120 125	
25	ggt ggt tcc gac ttc gac ccg aag ggc aag tcc gag ctg gag atc atg	432
	Gly Gly Ser Asp Phe Asp Pro Lys Gly Lys Ser Glu Leu Glu Ile Met	
	130 135 140	
30	cgc ttc tgc cag tcc ttc atg acc gag ctg cac cgc cac atc ggc gag	480
	Arg Phe Cys Gln Ser Phe Met Thr Glu Leu His Arg His Ile Gly Glu	
	145 150 155 160	
35	tac cgg gat gtc ccg gcc ggt gac atc gga gtc ggt ggc cgc gag atc	528
	Tyr Arg Asp Val Pro Ala Gly Asp Ile Gly Val Gly Gly Arg Glu Ile	
	165 170 175	
40	ggt tac ctc ttc ggc cac tac cgc cgt ctg gcc aac cag cac gag tcc	576
	Gly Tyr Leu Phe Gly His Tyr Arg Arg Leu Ala Asn Gln His Glu Ser	
	180 185 190	
45	ggt gtg ctc acc ggc aag ggc ctg acc tgg ggt ggt tcc ctg gtc cgc	624
	Gly Val Leu Thr Gly Lys Gly Leu Thr Trp Gly Gly Ser Leu Val Arg	
	195 200 205	
50	acc gag gcc acc ggc ttc ggc acc gtc tac ttc gtc cag gag atg atc	672
	Thr Glu Ala Thr Gly Phe Gly Thr Val Tyr Phe Val Gln Glu Met Ile	
	210 215 220	
55	aag cgc gaa ggg gag acc ctc gag ggc aag aag gtc atc gtc tcc ggt	720
	Lys Ala Glu Gly Glu Thr Leu Glu Gly Lys Lys Val Ile Val Ser Gly	
	225 230 235 240	
60	tcc ggc aac gtg gcc acc tac gcc atc cag aag gtg cag gaa ctg ggt	768
	Ser Gly Asn Val Ala Thr Tyr Ala Ile Gln Lys Val Gln Glu Leu Gly	
	245 250 255	
65	gcg gtt gtg gtc ggc ttc tcc gac tcc agc ggc tgg gtc tcc acc ccg	816
	Ala Val Val Val Gly Phe Ser Asp Ser Ser Gly Trp Val Ser Thr Pro	
	260 265 270	
70	aac ggt gtt gac gtg gcc aag ctg cgt gag atc aag gag gtc cgt cgt	864
	Asn Gly Val Asp Val Ala Lys Leu Arg Glu Ile Lys Glu Val Arg Arg	

	275	280	285	
	gca cgc glg tcc tcc tac gcc gac gag glg gag ggt gcg gag tac cac			912
5	Ala Arg Val Ser Ser Tyr Ala Asp Glu Val Glu Gly Ala Glu Tyr His			
	290	295	300	
	acc gac ggc tcc atc tgg gat ctg acc gcc gac atc gcg ctg ccc tgc			960
	Thr Asp Gly Ser Ile Trp Asp Leu Thr Ala Asp Ile Ala Leu Pro Cys			
10	305	310	315	320
	gcc acc cag aac gaa ctg gac ggc gac aac gcc cgc acc ctg gcg gac			1008
	Ala Thr Gln Asn Glu Leu Asp Gly Asp Asn Ala Arg Thr Leu Ala Asp			
	325	330	335	
15	aac ggc tgc cgc ttc gtg gcg gag ggc gcc aac atg ccc tcc acc ccc			1056
	Asn Gly Cys Arg Phe Val Ala Glu Gly Ala Asn Met Pro Ser Thr Pro			
	340	345	350	
	gag gcc atc gac gtc ttc cgt gag cgt ggt gtt ctg ttc ggg cgg ggc			1104
20	Glu Ala Ile Asp Val Phe Arg Glu Arg Gly Val Leu Phe Gly Pro Gly			
	355	360	365	
	aag gct gcc aac gcc ggt ggc gtg gcc acc tcc gcc ctg gag atg cag			1152
	Lys Ala Ala Asn Ala Gly Gly Val Ala Thr Ser Ala Leu Glu Met Gln			
25	370	375	380	
	cag aac gcc tcc cgt gat tcc tgg agc ttc gag tac acc gat gag cgt			1200
	Gln Asn Ala Ser Arg Asp Ser Trp Ser Phe Glu Tyr Thr Asp Glu Arg			
	385	390	395	400
30	ctc cac cgc atc atg aag aac atc ttc aag tcc tgc gcc gat acc gcc			1248
	Leu His Arg Ile Met Lys Asn Ile Phe Lys Ser Cys Ala Asp Thr Ala			
	405	410	415	
	aag gag tac ggc cac gag aag aac tac gtg gtc ggt gcg aac atc gcc			1296
35	Lys Glu Tyr Gly His Glu Lys Asn Tyr Val Val Gly Ala Asn Ile Ala			
	420	425	430	
	gga ttc aag aag gtc gct gac gcc atg ctg gcc cag ggt gtc atc taa			1344
	Gly Phe Lys Lys Val Ala Asp Ala Met Leu Ala Gln Gly Val Ile			
40	435	440	445	
	<210> 80			
	<211> 447			
45	<212> PRT			
	<213> Corynebacterium thermoaminogenes			
	<400> 80			
50	Met Thr Val Asp Glu Gln Val Ser Asn Tyr Tyr Asp Met Leu Leu Lys			
	1 5 10 15			
	Arg Asn Ala Gly Glu Pro Glu Phe His Gln Ala Val Ala Glu Val Leu			
	20 25 30			
55	Glu Ser Leu Lys Ile Val Leu Glu Lys Asp Pro His Tyr Ala Asp Tyr			

	35	40	45
	Gly	Leu	Ile
	50	55	60
5	Val	Pro	Trp
	65	70	75
	Arg	Val	Gln
10	85	90	95
	Phe	His	Pro
	100	105	110
	Gln	Ile	Phe
15	115	120	125
	Gly	Gly	Ser
	130	135	140
	Arg	Phe	Cys
20	145	150	155
	Tyr	Arg	Asp
	165	170	175
	Gly	Tyr	Leu
25	180	185	190
	Gly	Val	Leu
	195	200	205
	Thr	Glu	Ala
30	210	215	220
	Lys	Ala	Glu
	225	230	235
	Ser	Gly	Asn
35	245	250	255
	Ala	Val	Val
	260	265	270
	Asn	Gly	Val
40	275	280	285
	Ala	Arg	Val
	290	295	300
	Thr	Asp	Gly
45	305	310	315
	Ala	Thr	Gln
	325	330	335
	Asn	Gly	Cys
50	340	345	350
	Glu	Ala	Ile
	355	360	365
	Lys	Ala	Ala
55	370	375	380

Gln Asn Ala Ser Arg Asp Ser Trp Ser Phe Glu Tyr Thr Asp Glu Arg
 385 390 395 400

Leu His Arg Ile Met Lys Asn Ile Phe Lys Ser Cys Ala Asp Thr Ala
 405 410 415

Lys Glu Tyr Gly His Glu Lys Asn Tyr Val Val Gly Ala Asn Ile Ala
 420 425 430

Gly Phe Lys Lys Val Ala Asp Ala Met Leu Ala Gln Gly Val Ile
 435 440 445

<210> 81

<211> 1344

<212> DNA

<213> Brevibacterium lactofermentum

<220>

<221> CDS

<222> (1)..(1341)

<400> 81

atg aca gtt gat gag cag gtc tct aac tat tac gac atg ctt ctg aag 48

Met. Thr Val Asp Glu Gln Val Ser Asn Tyr Tyr Asp Met Leu Leu Lys

1 5 10 15

cgc aat gct ggc gag cct gaa ttt cac cag gca gtg gca gag gtt ttg 96

Arg Asn Ala Gly Glu Pro Glu Phe His Gln Ala Val Ala Glu Val Leu

20 25 30

gaa tct ttg aag atc gtc ctg gaa aag gac cct cat tac gct gat tac 144

Glu Ser Leu Lys Ile Val Leu Glu Lys Asp Pro His Tyr Ala Asp Tyr

35 40 45

ggt ctc atc cag cgc ctg tgc gag cct gag cgt cag ctc atc ttc cgt 192

Gly Leu Ile Gln Arg Leu Cys Glu Pro Glu Arg Gln Leu Ile Phe Arg

50 55 60

gtg cct lgg gtt gat gac cag ggc cag gtc cac gtc aac cgt ggt ttc 240

Val Pro Trp Val Asp Asp Gln Gly Gln Val His Val Asn Arg Gly Phe

65 70 75 80

cgc gtc cag ttc aac tct gca ctt gga cca tac aag ggc ggc ctg cgc 288

Arg Val Gln Phe Asn Ser Ala Leu Gly Pro Tyr Lys Gly Gly Leu Arg

85 90 95

ttc cac cca tct gta aac ctg ggc att gtg aag ttc ctg ggc ttt gag 336

Phe His Pro Ser Val Asn Leu Gly Ile Val Lys Phe Leu Gly Phe Glu

100 105 110

cag atc ttt aaa aac tcc cta acc ggc ctg cca atc ggt ggt ggc aag 384

Gln Ile Phe Lys Asn Ser Leu Thr Gly Leu Pro Ile Gly Gly Gly Lys

115 120 125

	ggt gga tcc gac ttc gac cct aag ggc aag tcc gat ctg gaa atc atg	432
	Gly Gly Ser Asp Phe Asp Pro Lys Gly Lys Ser Asp Leu Glu Ile Met	
5	130 135 140	
	cgt ttc tgc cag tcc ttc atg acc gag ctg cac cgc cac atc ggt gag	480
	Arg Phe Cys Gln Ser Phe Met Thr Glu Leu His Arg His Ile Gly Glu	
	145 150 155 160	
10	tac cgc gac gtt cct gca ggt gac atc gga gtt ggt ggc cgc gag atc	528
	Tyr Arg Asp Val Pro Ala Gly Asp Ile Gly Val Gly Gly Arg Glu Ile	
	165 170 175	
	ggt tac ctg ttt ggc cac tac cgt cgc atg gct aac cag cac gag tcc	576
15	Gly Tyr Leu Phe Gly His Tyr Arg Arg Met Ala Asn Gln His Glu Ser	
	180 185 190	
	ggc gtt ttg acc ggt aag ggc ctg acc tgg ggt gga tcc ctg gtc cgc	624
	Gly Val Leu Thr Gly Lys Gly Leu Thr Trp Gly Gly Ser Leu Val Arg	
20	195 200 205	
	acc gag gca act ggc tac ggc tgc gtt tac ttc gtg agt gaa atg atc	672
	Thr Glu Ala Thr Gly Tyr Gly Cys Val Tyr Phe Val Ser Glu Met Ile	
	210 215 220	
25	aag gct aag ggc gag agc atc agc ggc cag aag atc atc gtt tcc ggt	720
	Lys Ala Lys Gly Glu Ser Ile Ser Gly Gln Lys Ile Ile Val Ser Gly	
	225 230 235 240	
	tcc ggc aac gla gca acc tac gcg att gaa aag gct cag gaa ctg ggc	768
30	Ser Gly Asn Val Ala Thr Tyr Ala Ile Glu Lys Ala Gln Glu Leu Gly	
	245 250 255	
	gca acc gtt att ggt ttc tcc gat tcc agc ggt tgg gtt cat acc cct	816
	Ala Thr Val Ile Gly Phe Ser Asp Ser Ser Gly Trp Val His Thr Pro	
35	260 265 270	
	aac ggc gtt gac gtg gct aag ctg cgc gaa atc aag gaa gtt cgc cgc	864
	Asn Gly Val Asp Val Ala Lys Leu Arg Glu Ile Lys Glu Val Arg Arg	
	275 280 285	
40	gca cgc gla tcc gtg tac gcc gac gaa att gaa ggc gca acc tac cac	912
	Ala Arg Val Ser Val Tyr Ala Asp Glu Ile Glu Gly Ala Thr Tyr His	
	290 295 300	
45	acc gac ggt tcc atc tgg gat ctg aag tgc gat atc gct ctt cct tgt	960
	Thr Asp Gly Ser Ile Trp Asp Leu Lys Cys Asp Ile Ala Leu Pro Cys	
	305 310 315 320	
	gca act cag aac gag ctg aac ggc gag aac gct aag act ctt gca gac	1008
50	Ala Thr Gln Asn Glu Leu Asn Gly Glu Asn Ala Lys Thr Leu Ala Asp	
	325 330 335	
	aac ggc tgc cgt ttc gtt gct gaa ggc gcg aac atg cct tcc acc cct	1056
	Asn Gly Cys Arg Phe Val Ala Glu Gly Ala Asn Met Pro Ser Thr Pro	
55	340 345 350	
	gag gct gtt gag gtc ttc cgt gag cgc gac atc cgc ttc gga cca ggc	1104

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	Glu	Ala	Val	Glu	Val	Phe	Arg	Glu	Arg	Asp	Ile	Arg	Phe	Gly	Pro	Gly	
	355						360						365				
5	aag	gca	gct	aac	gct	ggt	ggc	ggt	gca	acc	tcc	gct	ctg	gag	atg	cag	1152
	Lys	Ala	Ala	Asn	Ala	Gly	Gly	Val	Ala	Thr	Ser	Ala	Leu	Glu	Met	Gln	
	370						375						380				
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	Gln	Asn	Ala	Ser	Arg	Asp	Ser	Trp	Ser	Phe	Glu	Tyr	Thr	Asp	Glu	Arg	
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	ctc	cag	gtg	atc	atg	aag	aac	atc	ttc	aag	acc	tgt	gca	gag	acc	gca	1248
	Leu	Gln	Val	Ile	Met	Lys	Asn	Ile	Phe	Lys	Thr	Cys	Ala	Glu	Thr	Ala	
15				405						410						415	
	gca	gag	tat	gga	cac	gag	aac	gat	tac	gtt	gtc	ggc	gct	aac	att	gct	1296
	Ala	Glu	Tyr	Gly	His	Glu	Asn	Asp	Tyr	Val	Val	Gly	Ala	Asn	Ile	Ala	
				420						425						430	
20	ggc	ttt	aag	aag	gta	gct	gac	gcg	atg	ctg	gca	cag	ggc	gtc	atc	taa	1344
	Gly	Phe	Lys	Lys	Val	Ala	Asp	Ala	Met	Leu	Ala	Gln	Gly	Val	Ile		
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	<211> 447																
	<212> PRT																
	<213> Brevibacterium lactofermentum																
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	20						25						30				
	Glu	Ser	Leu	Lys	Ile	Val	Leu	Glu	Lys	Asp	Pro	His	Tyr	Ala	Asp	Tyr	
	35						40						45				
40	Gly	Leu	Ile	Gln	Arg	Leu	Cys	Glu	Pro	Glu	Arg	Gln	Leu	Ile	Phe	Arg	
	50						55						60				
	Val	Pro	Trp	Val	Asp	Asp	Gln	Gly	Gln	Val	His	Val	Asn	Arg	Gly	Phe	
	65						70						75				
45	Arg	Val	Gln	Phe	Asn	Ser	Ala	Leu	Gly	Pro	Tyr	Lys	Gly	Gly	Leu	Arg	
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	Phe	His	Pro	Ser	Val	Asn	Leu	Gly	Ile	Val	Lys	Phe	Leu	Gly	Phe	Glu	
	100						105						110				
50	Gln	Ile	Phe	Lys	Asn	Ser	Leu	Thr	Gly	Leu	Pro	Ile	Gly	Gly	Gly	Lys	
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	Gly	Gly	Ser	Asp	Phe	Asp	Pro	Lys	Gly	Lys	Ser	Asp	Leu	Glu	Ile	Met	
	130						135						140				
55	Arg	Phe	Cys	Gln	Ser	Phe	Met	Thr	Glu	Leu	His	Arg	His	Ile	Gly	Glu	

145 150 155 160
 Tyr Arg Asp Val Pro Ala Gly Asp Ile Gly Val Gly Gly Arg Glu Ile
 165 170 175
 5 Gly Tyr Leu Phe Gly His Tyr Arg Arg Met Ala Asn Gln His Glu Ser
 180 185 190
 Gly Val Leu Thr Gly Lys Gly Leu Thr Trp Gly Gly Ser Leu Val Arg
 195 200 205
 10 Thr Glu Ala Thr Gly Tyr Gly Cys Val Tyr Phe Val Ser Glu Met Ile
 210 215 220
 Lys Ala Lys Gly Glu Ser Ile Ser Gly Gln Lys Ile Ile Val Ser Gly
 15 225 230 235 240
 Ser Gly Asn Val Ala Thr Tyr Ala Ile Glu Lys Ala Gln Glu Leu Gly
 245 250 255
 Ala Thr Val Ile Gly Phe Ser Asp Ser Ser Gly Trp Val His Thr Pro
 20 260 265 270
 Asn Gly Val Asp Val Ala Lys Leu Arg Glu Ile Lys Glu Val Arg Arg
 275 280 285
 Ala Arg Val Ser Val Tyr Ala Asp Glu Ile Glu Gly Ala Thr Tyr His
 25 290 295 300
 Thr Asp Gly Ser Ile Trp Asp Leu Lys Cys Asp Ile Ala Leu Pro Cys
 305 310 315 320
 Ala Thr Gln Asn Glu Leu Asn Gly Glu Asn Ala Lys Thr Leu Ala Asp
 30 325 330 335
 Asn Gly Cys Arg Phe Val Ala Glu Gly Ala Asn Met Pro Ser Thr Pro
 340 345 350
 Glu Ala Val Glu Val Phe Arg Glu Arg Asp Ile Arg Phe Gly Pro Gly
 35 355 360 365
 Lys Ala Ala Asn Ala Gly Gly Val Ala Thr Ser Ala Leu Glu Met Gln
 370 375 380
 Gln Asn Ala Ser Arg Asp Ser Trp Ser Phe Glu Tyr Thr Asp Glu Arg
 40 385 390 395 400
 Leu Gln Val Ile Met Lys Asn Ile Phe Lys Thr Cys Ala Glu Thr Ala

 405 410 415
 45 Ala Glu Tyr Gly His Glu Asn Asp Tyr Val Val Gly Ala Asn Ile Ala
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50 <210> 83

<211> 20

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55 <213> Artificial Sequence

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<223> Description of Artificial Sequence: primer for
amplifying gltA gene

<220>

<221> misc_feature

<222> (9)

<223> n=inosine

<400> 83

aagatcacnt acaatcgaygg

20

<210> 84

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer for
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<400> 84

tagaagtcta cgltcgggia

20

<210> 85

<211> 21

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: primer for
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21

<210> 86

<211> 21

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: primer for
amplifying gltA gene

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cggtaggaacc ggtgctgaca t

21

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<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: primer for
amplifying gltA gene

<400> 87

gggtgggga attcggatcg t

21

<210> 88

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer for
amplifying gltA gene

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tgtagtagcc gcgtagcg a

21

<210> 89

<211> 1293

<212> DNA

<213> Corynebacterium thermoaminogenes

<220>

<221> CDS

<222> (1).. (1290)

<400> 89

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10	ttc gag atg ggc atc aag cag gcc acc gag ggt aac tcc ggt gtc atc	96
	Phe Glu Met Gly Ile Lys Gln Ala Thr Glu Gly Asn Ser Gly Val Ile	
	20 25 30	
15	ctg ggt aag atg ctg tcg gaa acc ggt ctg gtc acc ttc gac ccc ggt	144
	Leu Gly Lys Met Leu Ser Glu Thr Gly Leu Val Thr Phe Asp Pro Gly	
	35 40 45	
20	tat gtc agc acc ggt tcc acc gaa tcc aag atc acc tac atc gat ggt	192
	Tyr Val Ser Thr Gly Ser Thr Glu Ser Lys Ile Thr Tyr Ile Asp Gly	
	50 55 60	
25	gat gca ggc atc ctg cgc tac cgc ggc tac gac att gcg gat ctg gcc	240
	Asp Ala Gly Ile Leu Arg Tyr Arg Gly Tyr Asp Ile Ala Asp Leu Ala	
	65 70 75 80	
30	gaa aat gcc acc ttc aat gag gtc tcc tac ctc ctg atc aag ggt gag	288
	Glu Asn Ala Thr Phe Asn Glu Val Ser Tyr Leu Leu Ile Lys Gly Glu	
	85 90 95	
35	ctc ccg acc ccg gaa gag ctc cac aag ttc aac gac gag att cgt cac	336
	Leu Pro Thr Pro Glu Glu Leu His Lys Phe Asn Asp Glu Ile Arg His	
	100 105 110	
40	cac acc ctg ctg gac gag gac ttc aag tcc cag ttc aat gtc ttc cct	384
	His Thr Leu Leu Asp Glu Asp Phe Lys Ser Gln Phe Asn Val Phe Pro	
	115 120 125	
45	cgc gat gcc cac ccg atg gcc acc ctg gcc tcc tcg gtt aac atc ctc	432
	Arg Asp Ala His Pro Met Ala Thr Leu Ala Ser Ser Val Asn Ile Leu	
	130 135 140	
50	tcc acc tac tac cag gat cag ctg gat ccc ctg gat gag gct cag ctg	480
	Ser Thr Tyr Tyr Gln Asp Gln Leu Asp Pro Leu Asp Glu Ala Gln Leu	
	145 150 155 160	
55	gac aag gca acc gtc cgc ctg atg gcg aag gtt ccg atg ctg gct gca	528
	Asp Lys Ala Thr Val Arg Leu Met Ala Lys Val Pro Met Leu Ala Ala	
	165 170 175	
60	tac gca cac cgt gcc cgc aag ggt gcg ccg tac atg tac ccg gac aac	576
	Tyr Ala His Arg Ala Arg Lys Gly Ala Pro Tyr Met Tyr Pro Asp Asn	
	180 185 190	
65	tcc ctc aat gcc cgt gag aac ttc ctg cgc atg atg ttc ggt tac ccg	624
	Ser Leu Asn Ala Arg Glu Asn Phe Leu Arg Met Met Phe Gly Tyr Pro	
	195 200 205	
70	acc gag ccg tac gag gtt gat ccg atc atg gtc aaa gcc ctc gac aag	672
	Thr Glu Pro Tyr Glu Val Asp Pro Ile Met Val Lys Ala Leu Asp Lys	

210 215 220
 5 ctc ctc atc ctg cac gca gac cac gag cag aac tgc tcc acc tcc act 720
 Leu Leu Ile Leu His Ala Asp His Glu Gln Asn Cys Ser Thr Ser Thr
 225 230 235 240
 gtc cgc atg atc ggc tcc gcg cag gcg aac atg ttc gtc tcc atc gcc 768
 Val Arg Met Ile Gly Ser Ala Gln Ala Asn Met Phe Val Ser Ile Ala
 10 245 250 255
 ggc ggc atc aac gca ctc tcc ggc cgc ctg cac ggt ggc gcc aac cag 816
 Gly Gly Ile Asn Ala Leu Ser Gly Pro Leu His Gly Gly Ala Asn Gln
 260 265 270
 15 gct gtc ctc gag atg ctc gag gag atc gca gcc aac ggc ggc gac gca 864
 Ala Val Leu Glu Met Leu Glu Glu Ile Ala Ala Asn Gly Gly Asp Ala
 275 280 285
 20 acc gac ttc atg aac cgc gtc aag aac aag gag aag ggt gtc cgc ctc 912
 Thr Asp Phe Met Asn Arg Val Lys Asn Lys Glu Lys Gly Val Arg Leu
 290 295 300
 atg ggc ttc gga cac cgc gtc tac aag aac tac gat cgc cgt gca gcc 960
 Met Gly Phe Gly His Arg Val Tyr Lys Asn Tyr Asp Pro Arg Ala Ala
 25 305 310 315 320
 atc gtc aag gac acc gcc cac gag atc ctc gag cac ctc ggt ggc gac 1008
 Ile Val Lys Asp Thr Ala His Glu Ile Leu Glu His Leu Gly Gly Asp
 325 330 335
 30 cca ctg ctg gat ctg gct ctc aag ctg gaa gaa atc gca ctc aac gac 1056
 Pro Leu Leu Asp Leu Ala Leu Lys Leu Glu Glu Ile Ala Leu Asn Asp
 340 345 350
 35 gat tac ttc atc tcc cgc aag ctg tac cgc aac gtc gac ttc tac acc 1104
 Asp Tyr Phe Ile Ser Arg Lys Leu Tyr Pro Asn Val Asp Phe Tyr Thr
 355 360 365
 ggc ctg atc tac cgc gcc atg ggc ttc cgc acg gac ttc ttc acc gtc 1152
 Gly Leu Ile Tyr Arg Ala Met Gly Phe Pro Thr Asp Phe Phe Thr Val
 40 370 375 380
~~ctc ttc gcc atc ggc cgc ctc cgc ggc tgc atc gcc cac tac cgc gag~~ 1200
 Leu Phe Ala Ile Gly Arg Leu Pro Gly Trp Ile Ala His Tyr Arg Glu
 385 390 395 400
 45 cag ctc gcc gat cgc ggc gcc aag atc aac cgt cct cgc cag atc tac 1248
 Gln Leu Ala Asp Pro Gly Ala Lys Ile Asn Arg Pro Arg Gln Ile Tyr
 405 410 415
 50 acc ggt gag acc gca cgc aag atc atc ccc cgc gaa gag cgc tag 1293
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 420 425 430
 55 <210> 90
 <211> 430

<212> PRT

<213> *Corynebacterium thermoaminogenes*

<400> 90

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 20 25 30
 Leu Gly Lys Met Leu Ser Glu Thr Gly Leu Val Thr Phe Asp Pro Gly
 35 40 45
 Tyr Val Ser Thr Gly Ser Thr Glu Ser Lys Ile Thr Tyr Ile Asp Gly
 50 55 60
 Asp Ala Gly Ile Leu Arg Tyr Arg Gly Tyr Asp Ile Ala Asp Leu Ala
 65 70 75 80
 Glu Asn Ala Thr Phe Asn Glu Val Ser Tyr Leu Leu Ile Lys Gly Glu
 85 90 95
 Leu Pro Thr Pro Glu Glu Leu His Lys Phe Asn Asp Glu Ile Arg His
 100 105 110
 His Thr Leu Leu Asp Glu Asp Phe Lys Ser Gln Phe Asn Val Phe Pro
 115 120 125
 Arg Asp Ala His Pro Met Ala Thr Leu Ala Ser Ser Val Asn Ile Leu
 130 135 140
 Ser Thr Tyr Tyr Gln Asp Gln Leu Asp Pro Leu Asp Glu Ala Gln Leu
 145 150 155 160
 Asp Lys Ala Thr Val Arg Leu Met Ala Lys Val Pro Met Leu Ala Ala
 165 170 175
 Tyr Ala His Arg Ala Arg Lys Gly Ala Pro Tyr Met Tyr Pro Asp Asn
 180 185 190
 Ser Leu Asn Ala Arg Glu Asn Phe Leu Arg Met Met Phe Gly Tyr Pro
 195 200 205
 Thr Glu Pro Tyr Glu Val Asp Pro Ile Met Val Lys Ala Leu Asp Lys
 210 215 220
 Leu Leu Ile Leu His Ala Asp His Glu Gln Asn Cys Ser Thr Ser Thr
 225 230 235 240
 Val Arg Met Ile Gly Ser Ala Gln Ala Asn Met Phe Val Ser Ile Ala
 245 250 255
 Gly Gly Ile Asn Ala Leu Ser Gly Pro Leu His Gly Gly Ala Asn Gln
 260 265 270
 Ala Val Leu Glu Met Leu Glu Glu Ile Ala Ala Asn Gly Gly Asp Ala
 275 280 285
 Thr Asp Phe Met Asn Arg Val Lys Asn Lys Glu Lys Gly Val Arg Leu
 290 295 300
 Met Gly Phe Gly His Arg Val Tyr Lys Asn Tyr Asp Pro Arg Ala Ala

305 310 315 320
 Ile Val Lys Asp Thr Ala His Glu Ile Leu Glu His Leu Gly Gly Asp
 325 330 335
 5 Pro Leu Leu Asp Leu Ala Leu Lys Leu Glu Glu Ile Ala Leu Asn Asp
 340 345 350
 Asp Tyr Phe Ile Ser Arg Lys Leu Tyr Pro Asn Val Asp Phe Tyr Thr
 355 360 365
 10 Gly Leu Ile Tyr Arg Ala Met Gly Phe Pro Thr Asp Phe Phe Thr Val
 370 375 380
 Leu Phe Ala Ile Gly Arg Leu Pro Gly Trp Ile Ala His Tyr Arg Glu
 385 390 395 400
 15 Gln Leu Ala Asp Pro Gly Ala Lys Ile Asn Arg Pro Arg Gln Ile Tyr
 405 410 415
 Thr Gly Glu Thr Ala Arg Lys Ile Ile Pro Arg Glu Glu Arg
 420 425 430
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<210> 91

<211> 1314

<212> DNA

<213> Brevibacterium lactofermentum

<220>

<221> CDS

<222> (1)..(1311)

<400> 91

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 40 cac tac ccc ggt ggc gag ttc gaa atg gac atc atc gag gct tct gag 96
 His Tyr Pro Gly Gly Glu Phe Glu Met Asp Ile Ile Glu Ala Ser Glu
 20 25 30

 45 ggt aac aac ggt gtt gtc ctg ggc aag atg ctg tct gag act gga ctg 144
 Gly Asn Asn Gly Val Val Leu Gly Lys Met Leu Ser Glu Thr Gly Leu
 35 40 45
 atc act ttt gac cca ggt tat gtg agc act ggc tcc acc gag tcc aag 192
 Ile Thr Phe Asp Pro Gly Tyr Val Ser Thr Gly Ser Thr Glu Ser Lys
 50 55 60
 50 atc acc tac atc gat ggc gat gcg gga atc ctg cgt tac cgc ggc tat 240
 Ile Thr Tyr Ile Asp Gly Asp Ala Gly Ile Leu Arg Tyr Arg Gly Tyr
 65 70 75 80
 55 gac atc gct gat ctg gct gag aat gcc acc ttc aac gag gtt tct tac 288
 Asp Ile Ala Asp Leu Ala Glu Asn Ala Thr Phe Asn Glu Val Ser Tyr

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		85		90		95	
		cla ctt atc aac ggt gaa cta cca acc cca gat gag ctt cac aag ttt	336				
5		Leu Leu Ile Asn Gly Glu Leu Pro Thr Pro Asp Glu Leu His Lys Phe					
		100 105 110					
		aac gac gag att cgc cac cac acc ctt ctg gac gag gac ttc aag tcc	384				
		Asn Asp Glu Ile Arg His His Thr Leu Leu Asp Glu Asp Phe Lys Ser					
		115 120 125					
10		cag ttc aac gtg ttc cca cgc gac gct cac cca atg gca acc ttg gct	432				
		Gln Phe Asn Val Phe Pro Arg Asp Ala His Pro Met Ala Thr Leu Ala					
		130 135 140					
15		tcc tct gtt aac att ttg tct acc tac tac cag gat cag ctg aac cca	480				
		Ser Ser Val Asn Ile Leu Ser Thr Tyr Tyr Gln Asp Gln Leu Asn Pro					
		145 150 155 160					
		ctc gat gag gca cag ctt gat aag gca acc gtt cgc ctc atg gca aag	528				
20		Leu Asp Glu Ala Gln Leu Asp Lys Ala Thr Val Arg Leu Met Ala Lys					
		165 170 175					
		gtt cca atg ctg gct gcg tac gca cac cgc gca cgc aag ggt gct cct	576				
		Val Pro Met Leu Ala Ala Tyr Ala His Arg Ala Arg Lys Gly Ala Pro					
		180 185 190					
25		tac atg tac cca gac aac tcc ctc aac gcg cgt gag aac ttc ctg cgc	624				
		Tyr Met Tyr Pro Asp Asn Ser Leu Asn Ala Arg Glu Asn Phe Leu Arg					
		195 200 205					
30		atg atg ttc ggt tac cca acc gag cca tac gag atc gac cca atc atg	672				
		Met Met Phe Gly Tyr Pro Thr Glu Pro Tyr Glu Ile Asp Pro Ile Met					
		210 215 220					
		gtc aag gct ctg gac aag ctg ctc atc ctg cac gct gac cac gag cag	720				
35		Val Lys Ala Leu Asp Lys Leu Leu Ile Leu His Ala Asp His Glu Gln					
		225 230 235 240					
		aac tgc tcc acc tcc acc gtt cgt atg atc ggt tcc gca cag gcc aac	768				
		Asn Cys Ser Thr Ser Thr Val Arg Met Ile Gly Ser Ala Gln Ala Asn					
40		245 250 255					
		atg ttt gtc tcc atc gct ggt ggc atc aac gct ctg tcc ggc cca ctg	816				
		Met Phe Val Ser Ile Ala Gly Gly Ile Asn Ala Leu Ser Gly Pro Leu					
		260 265 270					
45		cac ggt ggc gca aac cag gct gtt ctg gag atg ctc gaa gac atc aag	864				
		His Gly Gly Ala Asn Gln Ala Val Leu Glu Met Leu Glu Asp Ile Lys					
		275 280 285					
		aac aac cac ggt ggc gac gca acc gcg ttc atg aac aag gtc aag aac	912				
50		Asn Asn His Gly Gly Asp Ala Thr Ala Phe Met Asn Lys Val Lys Asn					
		290 295 300					
		aag gaa gac ggc gtc cgc ctc atg ggc ttc gga cac cgc gtt tac aag	960				
		Lys Glu Asp Gly Val Arg Leu Met Gly Phe Gly His Arg Val Tyr Lys					
55		305 310 315 320					

aac tac gal cca cgt gca gca atc gtc aag gag acc gca cac gag atc 1008
 Asn Tyr Asp Pro Arg Ala Ala Ile Val Lys Glu Thr Ala His Glu Ile
 325 330 335
 5 ctc gag cac ctc ggt ggc gac gal ctt ctg gal ctg gca atc aag ctg 1056
 Leu Glu His Leu Gly Gly Asp Asp Leu Leu Asp Leu Ala Ile Lys Leu
 340 345 350
 10 gaa gaa att gca ctg gct gal gal tac ttc atc tcc cgc aag ctc tac 1104
 Glu Glu Ile Ala Leu Ala Asp Asp Tyr Phe Ile Ser Arg Lys Leu Tyr
 355 360 365
 15 ccg aac gta gac ttc tac acc ggc ctg atc tac cgc gca atg ggc ttc 1152
 Pro Asn Val Asp Phe Tyr Thr Gly Leu Ile Tyr Arg Ala Met Gly Phe
 370 375 380
 cca act gac ttc ttc acc gta ttg ttc gca atc ggt cgt ctg cca gga 1200
 Pro Thr Asp Phe Phe Thr Val Leu Phe Ala Ile Gly Arg Leu Pro Gly
 385 390 395 400
 20 tgg atc gct cac tac cgc gag cag ctg ggt gca gca ggc aac aag atc 1248
 Trp Ile Ala His Tyr Arg Glu Gln Leu Gly Ala Ala Gly Asn Lys Ile
 405 410 415
 25 aac cgc cca cgc cag gtc tac acc ggc aag gaa tcc cgc aag ttg gtt 1296
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<210> 92

<211> 437

<212> PRT

<213> Brevibacterium lactofermentum

<400> 92

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 35 40 45
 50 Ile Thr Phe Asp Pro Gly Tyr Val Ser Thr Gly Ser Thr Glu Ser Lys
 50 55 60
 Ile Thr Tyr Ile Asp Gly Asp Ala Gly Ile Leu Arg Tyr Arg Gly Tyr
 65 70 75 80
 55 Asp Ile Ala Asp Leu Ala Glu Asn Ala Thr Phe Asn Glu Val Ser Tyr
 85 90 95

	Leu	Leu	Ile	Asn	Gly	Glu	Leu	Pro	Thr	Pro	Asp	Glu	Leu	His	Lys	Phe
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	Gln	Phe	Asn	Val	Phe	Pro	Arg	Asp	Ala	His	Pro	Met	Ala	Thr	Leu	Ala
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10	Ser	Ser	Val	Asn	Ile	Leu	Ser	Thr	Tyr	Tyr	Gln	Asp	Gln	Leu	Asn	Pro
	145					150					155				160	
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					165					170					175	
15	Val	Pro	Met	Leu	Ala	Ala	Tyr	Ala	His	Arg	Ala	Arg	Lys	Gly	Ala	Pro
			180					185						190		
	Tyr	Met	Tyr	Pro	Asp	Asn	Ser	Leu	Asn	Ala	Arg	Glu	Asn	Phe	Leu	Arg
		195					200						205			
20	Met	Met	Phe	Gly	Tyr	Pro	Thr	Glu	Pro	Tyr	Glu	Ile	Asp	Pro	Ile	Met
		210					215					220				
	Val	Lys	Ala	Leu	Asp	Lys	Leu	Leu	Ile	Leu	His	Ala	Asp	His	Glu	Gln
	225					230					235				240	
25	Asn	Cys	Ser	Thr	Ser	Thr	Val	Arg	Met	Ile	Gly	Ser	Ala	Gln	Ala	Asn
				245						250					255	
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			260						265					270		
30	His	Gly	Gly	Ala	Asn	Gln	Ala	Val	Leu	Glu	Met	Leu	Glu	Asp	Ile	Lys
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		290					295					300				
35	Lys	Glu	Asp	Gly	Val	Arg	Leu	Met	Gly	Phe	Gly	His	Arg	Val	Tyr	Lys
	305					310					315				320	
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40	Leu	Glu	His	Leu	Gly	Gly	Asp	Asp	Leu	Leu	Asp	Leu	Ala	Ile	Lys	Leu
			340						345					350		
	Glu	Glu	Ile	Ala	Leu	Ala	Asp	Asp	Tyr	Phe	Ile	Ser	Arg	Lys	Leu	Tyr
		355						360					365			
45	Pro	Asn	Val	Asp	Phe	Tyr	Thr	Gly	Leu	Ile	Tyr	Arg	Ala	Met	Gly	Phe
		370					375					380				
	Pro	Thr	Asp	Phe	Phe	Thr	Val	Leu	Phe	Ala	Ile	Gly	Arg	Leu	Pro	Gly
	385					390					395				400	
50	Trp	Ile	Ala	His	Tyr	Arg	Glu	Gln	Leu	Gly	Ala	Ala	Gly	Asn	Lys	Ile
				405						410					415	
	Asn	Arg	Pro	Arg	Gln	Val	Tyr	Thr	Gly	Lys	Glu	Ser	Arg	Lys	Leu	Val
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55	Pro	Arg	Glu	Glu	Arg											

435

<210> 93

<211> 1656

<212> DNA

<213> Corynebacterium thermoaminogenes

<220>

<221> CDS

<222> (309).. (1595)

<400> 93

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Met His Thr Glu Leu Ser Ser Leu Arg Pro Ala Tyr His Val
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act cct ccg cag ggc aga ctc aat gat ccc aat gga atg tac gtc gat 398
Thr Pro Pro Gln Gly Arg Leu Asn Asp Pro Asn Gly Met Tyr Val Asp
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gga gat acc ctc cac glc tac tac cag cac gat cca ggt ttc ccc ttc 446
Gly Asp Thr Leu His Val Tyr Tyr Gln His Asp Pro Gly Phe Pro Phe
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Ala Pro Lys Arg Thr Gly Trp Ala His Thr Thr Thr Pro Leu Thr Gly
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Pro Gln Arg Leu Gln Trp Thr His Leu Pro Asp Ala Leu Tyr Pro Asp
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Val Ser Tyr Asp Leu Asp Gly Cys Tyr Ser Gly Gly Ala Val Phe Ser
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Lys Arg Arg Ala Thr Gln Asn Leu Val Glu Val Glu Asp Pro Thr Gly
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	ctc acc ggt gca gcg gtt cta tac cgc tcg gca gat ctt gaa aac tgg	878		
	Leu Thr Gly Ala Ala Val Leu Tyr Arg Ser Ala Asp Leu Glu Asn Trp			
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 40 65 70 75 80
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 85 90 95
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 45 100 105 110
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30

Claims

1. A protein having the amino acid sequence of SEQ ID NO: 2 or the amino acid sequence of SEQ ID NO: 2 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has isocitrate lyase activity and shows 30% or more of residual activity after a heat treatment at 50°C for 5 minutes.
2. A protein having the amino acid sequence of SEQ ID NO: 4 or the amino acid sequence of SEQ ID NO: 4 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which is involved in acyl Co-A carboxylase activity and is derived from *Corynebacterium thermoaminogenes*.
3. A protein having the amino acid sequence of SEQ ID NO: 6 or the amino acid sequence of SEQ ID NO: 6 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has DtsR activity and is derived from *Corynebacterium thermoaminogenes*.
4. A protein having the amino acid sequence of SEQ ID NO: 8 or the amino acid sequence of SEQ ID NO: 8 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has DtsR activity and is derived from *Corynebacterium thermoaminogenes*.
5. A protein having the amino acid sequence of SEQ ID NO: 10 or the amino acid sequence of SEQ ID NO: 10 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which shows phosphofructokinase activity at 60°C in an equivalent or higher degree compared with the activity at 30°C.
6. A protein having the amino acid sequence of SEQ ID NO: 94 or the amino acid sequence of SEQ ID NO: 94 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has activity for imparting sucrose assimilating ability to *Corynebacterium thermoaminogenes*.
7. A protein having any one of the amino acid sequences of SEQ ID NOS: 17-20 or the amino acid sequence of any one of SEQ ID NOS: 17-20 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has a function involved in glutamic acid uptake and is derived from *Corynebacterium thermoaminogenes*.
8. A protein having the amino acid sequence of SEQ ID NO: 22 or the amino acid sequence of SEQ ID NO: 22 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has pyruvate dehydrogenase activity and is derived from *Corynebacterium thermoaminogenes*.
9. A protein having the amino acid sequence of SEQ ID NO: 24 or the amino acid sequence of SEQ ID NO: 24 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has pyruvate carboxylase activity and is derived from *Corynebacterium thermoaminogenes*.
10. A protein having the amino acid sequence of SEQ ID NO: 26 or the amino acid sequence of SEQ ID NO: 26 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has phosphoenolpyruvate carboxylase activity and shows 50% or more of residual activity after a heat treatment at 45°C for 5 minutes.
11. A protein having the amino acid sequence of SEQ ID NO: 28 or the amino acid sequence of SEQ ID NO: 28 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has aconitase activity and shows 30% or more of residual activity after a heat treatment at 50°C for 3 minutes.
12. A protein having the amino acid sequence of SEQ ID NO: 30 or the amino acid sequence of SEQ ID NO: 30 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has isocitrate dehydrogenase activity and shows 50% or more of residual activity after a heat treatment at 45°C for 10 minutes.
13. A protein having the amino acid sequence of SEQ ID NO: 32 or the amino acid sequence of SEQ ID NO: 32 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has dihydroipoamide dehydrogenase activity and is derived from *Corynebacterium thermoaminogenes*.
14. A protein having the amino acid sequence of SEQ ID NO: 34 or the amino acid sequence of SEQ ID NO: 34

including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which has 2-oxoglutarate dehydrogenase activity and shows 30% or more of residual activity after a heat treatment at 50°C for 10 minutes.

- 5 15. A protein having the amino acid sequence of SEQ ID NO: 80 in Sequence Listing or the amino acid sequence of SEQ ID NO: 80 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which shows glutamate dehydrogenase activity at 42°C in an equivalent or higher degree compared with the activity at 37°C.
- 10 16. A protein having the amino acid sequence of SEQ ID NO: 90 in Sequence Listing or the amino acid sequence of SEQ ID NO: 90 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, which shows citrate synthase activity at 37°C in an equivalent or higher degree compared with the activity at 23°C.
- 15 17. A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 2 or the amino acid sequence of SEQ ID NO: 2 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having isocitrate lyase activity.
- 20 18. The DNA according to Claim 17, which is a DNA defined in the following (a1) or (b1):
 - (a1) a DNA which comprises the nucleotide sequence of SEQ ID NO: 1 in Sequence Listing,
 - (b1) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 1 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having isocitrate lyase activity.
- 25 19. A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 4 or the amino acid sequence of SEQ ID NO: 4 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and involved in acyl Co-A carboxylase activity.
- 30 20. The DNA according to Claim 19, which is a DNA defined in the following (a2) or (b2):
 - (a2) a DNA which comprises the nucleotide sequence of SEQ ID NO: 3 in Sequence Listing,
 - (b2) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 3 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein involved in acyl Co-A carboxylase activity.
- 35 21. A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 6 or the amino acid sequence of SEQ ID NO: 6 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having DtsR activity.
- 40 22. The DNA according to Claim 21, which is a DNA defined in the following (a3) or (b3):
 - (a3) a DNA which comprises the nucleotide sequence of SEQ ID NO: 5 in Sequence Listing,
 - (b3) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 5 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having DtsR activity.
- 45 23. A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 8 or the amino acid sequence of SEQ ID NO: 8 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having DtsR activity.
- 50 24. The DNA according to Claim 23, which is a DNA defined in the following (a4) or (b4):
 - (a4) a DNA which comprises the nucleotide sequence of SEQ ID NO: 7 in Sequence Listing,
 - (b4) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 7 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having DtsR activity.
- 55

25. A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 10 or the amino acid sequence of SEQ ID NO: 10 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having phosphofructokinase activity.
26. The DNA according to Claim 25, which is a DNA defined in the following (a5) or (b5):
- (a5) a DNA which comprises the nucleotide sequence of SEQ ID NO: 9 in Sequence Listing,
 (b5) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 9 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having phosphofructokinase activity.
27. A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 93 or the amino acid sequence of SEQ ID NO: 93 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having invertase activity.
28. The DNA according to Claim 27, which is a DNA defined in the following (a6) or (b6):
- (a6) a DNA which comprises the nucleotide sequence of SEQ ID NO: 93 in Sequence Listing,
 (b6) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 93 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having invertase activity.
29. A DNA which codes for a protein having any one of the amino acid sequences of SEQ ID NOS: 17-20 or the amino acid sequence of any one of SEQ ID NOS: 17-20 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having a function involved in glutamic acid uptake.
30. The DNA according to Claim 29, which is a DNA defined in the following (a7) or (b7):
- (a7) a DNA which comprises the nucleotide sequence of SEQ ID NO: 16 in Sequence Listing,
 (b7) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 16 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having a function involved in glutamic acid uptake.
31. A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 22 or the amino acid sequence of SEQ ID NO: 22 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having pyruvate dehydrogenase activity.
32. The DNA according to Claim 31, which is a DNA defined in the following (a8) or (b8):
- (a8) a DNA which comprises the nucleotide sequence of SEQ ID NO: 21 in Sequence Listing,
 (b8) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 21 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having pyruvate dehydrogenase activity.
33. A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 24 or the amino acid sequence of SEQ ID NO: 24 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having pyruvate carboxylase activity.
34. A DNA according to Claim 33, which is a DNA defined in the following (a9) or (b9):
- (a9) a DNA which comprises the nucleotide sequence of SEQ ID NO: 23 in Sequence Listing,
 (b9) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 23 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having pyruvate carboxylase activity.
35. A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 26 or the amino acid sequence of SEQ ID NO: 26 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having phosphoenolpyruvate carboxylase activity.

36. The DNA according to Claim 35, which is a DNA defined in the following (a10) or (b10):

(a10) a DNA which comprises the nucleotide sequence of SEQ ID NO: 25 in Sequence Listing

(b10) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 25 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having phosphoenolpyruvate carboxylase activity.

37. A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 28 or the amino acid sequence of SEQ ID NO: 28 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having aconitase activity.

38. The DNA according to Claim 37, which is a DNA defined in the following (a11) or (b11):

(a11) a DNA which comprises the nucleotide sequence of SEQ ID NO: 27 in Sequence Listing.

(b11) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 27 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having aconitase activity.

39. A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 30 or the amino acid sequence of SEQ ID NO: 30 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having isocitrate dehydrogenase activity.

40. The DNA according to Claim 39, which is a DNA defined in the following (a12) or (b12):

(a12) a DNA which comprises the nucleotide sequence of SEQ ID NO: 27 in Sequence Listing.

(b12) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 27 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having isocitrate dehydrogenase activity.

41. A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 32 or the amino acid sequence of SEQ ID NO: 32 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having dihydrolipoamide dehydrogenase activity.

42. The DNA according to Claim 41, which is a DNA defined in the following (a13) or (b13):

(a13) a DNA which comprises the nucleotide sequence of SEQ ID NO: 31 in Sequence Listing.

(b13) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 31 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having dihydrolipoamide dehydrogenase activity.

43. A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 34 or the amino acid sequence of SEQ ID NO: 34 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and having 2-oxoglutarate dehydrogenase activity.

44. The DNA according to Claim 43, which is a DNA defined in the following (a14) or (b14):

(a14) a DNA which comprises the nucleotide sequence of SEQ ID NO: 33 in Sequence Listing.

(b14) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 33 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein having 2-oxoglutarate dehydrogenase activity.

45. A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 80 in Sequence Listing or the amino acid sequence of SEQ ID NO: 80 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and showing glutamate dehydrogenase activity at 42°C in an equivalent or higher degree compared with the activity at 37°C.

46. The DNA according to Claim 45, which is a DNA defined in the following (a15) or (b15):

(a15) a DNA which comprises the nucleotide sequence of SEQ ID NO: 79 in Sequence Listing,

(b15) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 79 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein showing glutamate dehydrogenase activity at 42°C in an equivalent or higher degree compared with the activity at 37°C.

47. A DNA which codes for a protein having the amino acid sequence of SEQ ID NO: 90 in Sequence Listing or the amino acid sequence of SEQ ID NO: 90 including substitution, deletion, insertion, addition or inversion of one or several amino acids residues, and showing citrate synthase activity at 37°C in an equivalent or higher degree compared with the activity at 23°C.

48. The DNA according to Claims 47, which is a DNA defined in the following (a16) or (b16):

(a16) a DNA which comprises the nucleotide sequence of SEQ ID NO: 89 in Sequence Listing,

(b16) a DNA which is hybridizable with the nucleotide sequence of SEQ ID NO: 89 in Sequence Listing or a primer prepared based on the nucleotide sequence under a stringent condition, and codes for a protein showing citrate synthase activity at 37°C in an equivalent or higher degree compared with the activity at 23°C.

49. A method for producing L-amino acid, which comprises culturing a microorganism introduced with a DNA according to any one of Claims 17 to 48 in a medium to produce and accumulate L-amino acid in the medium, and collecting the L-amino acid from the medium.

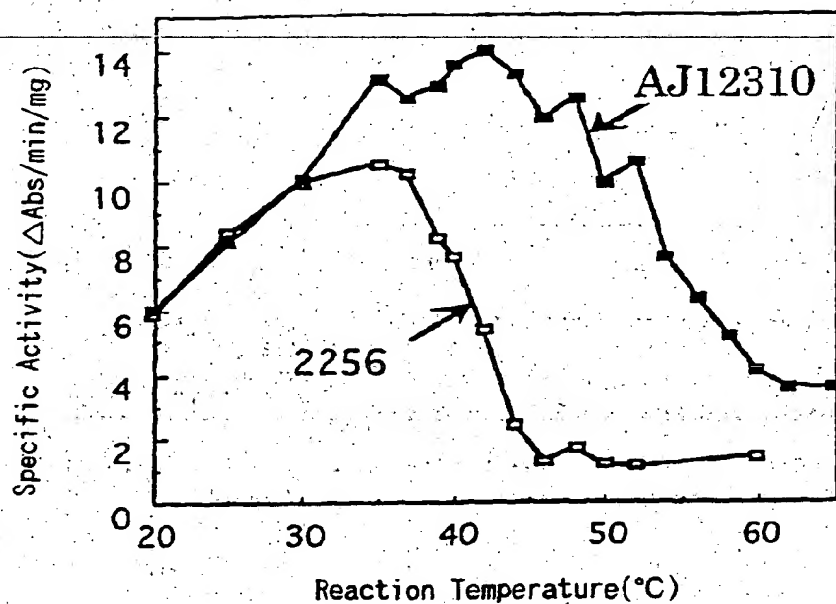


Fig. 1

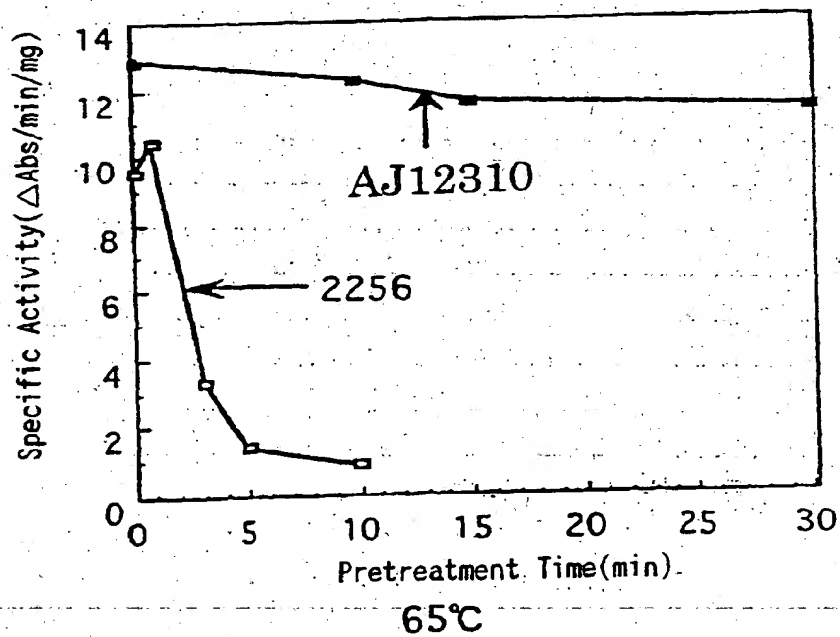


Fig. 2

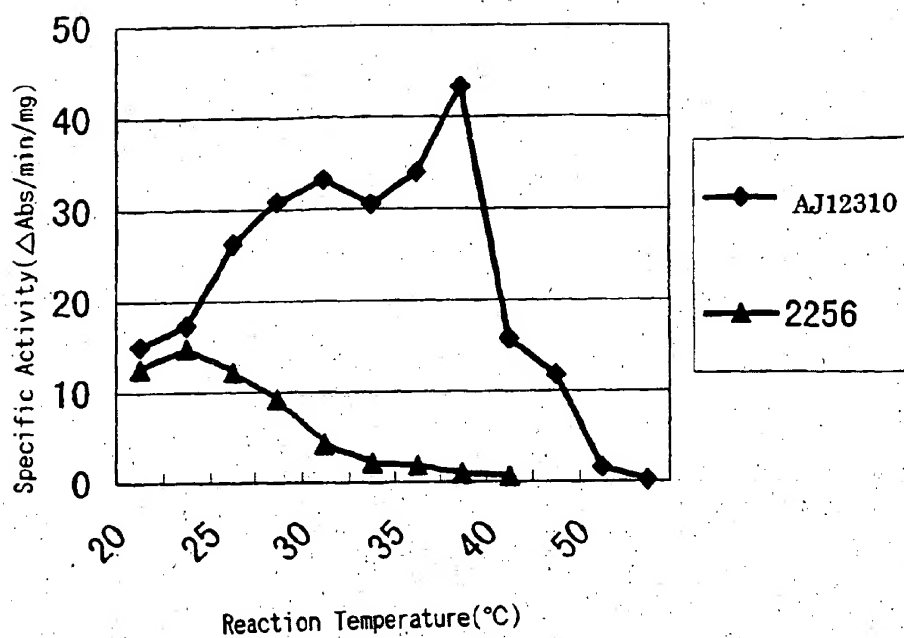


Fig. 3

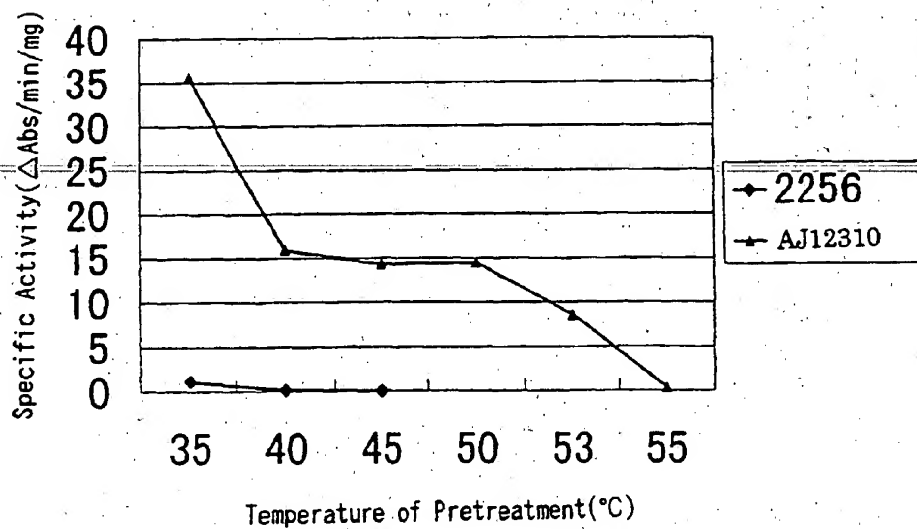


Fig. 4

Fig. 5

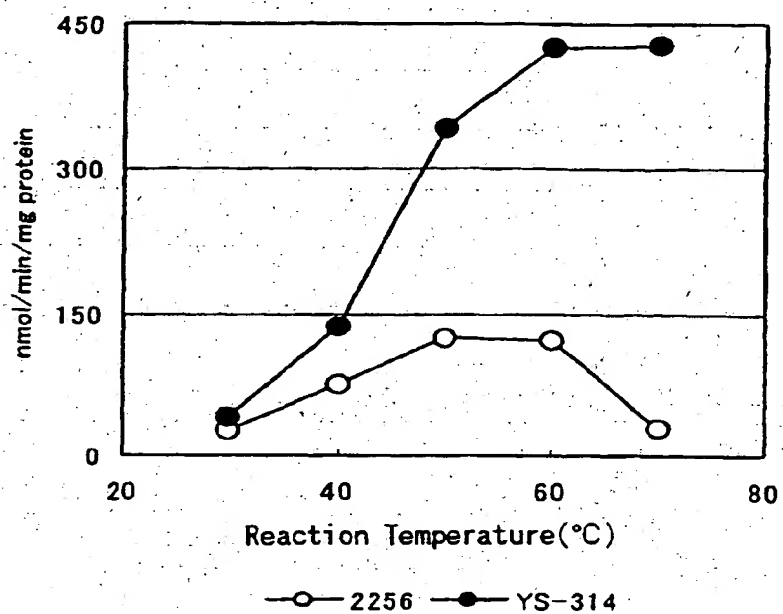


Fig. 6

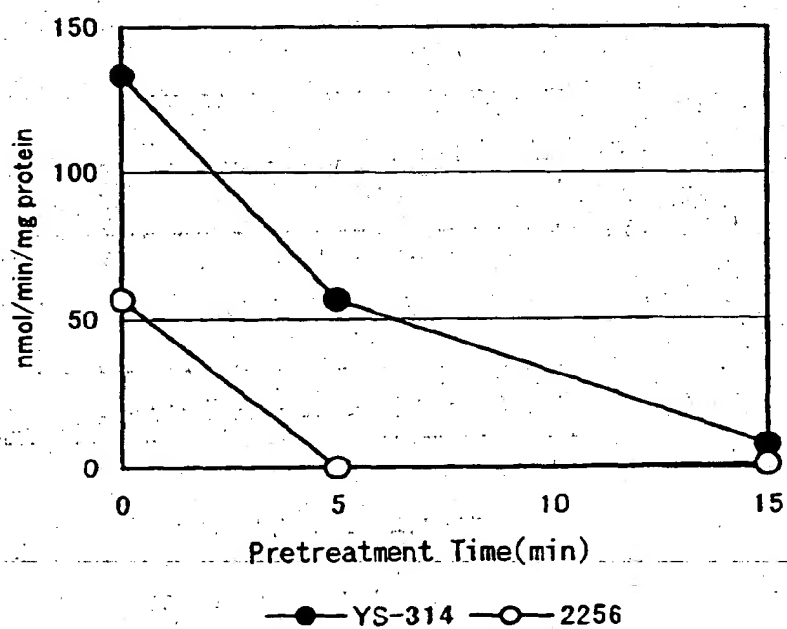


Fig. 7

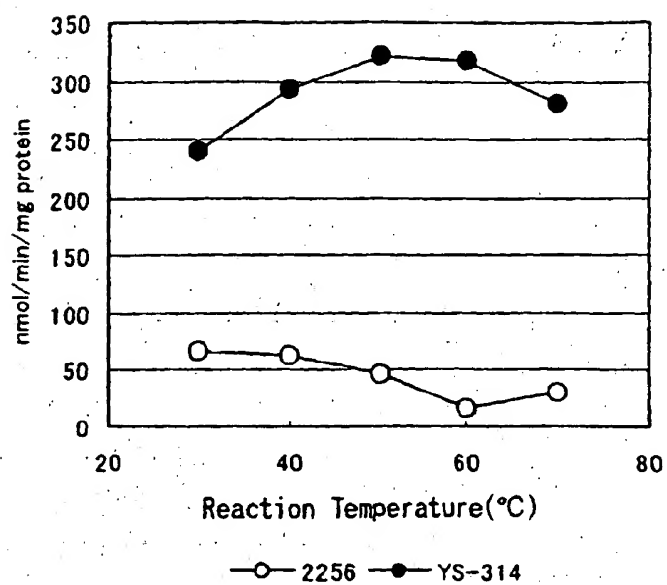


Fig. 8

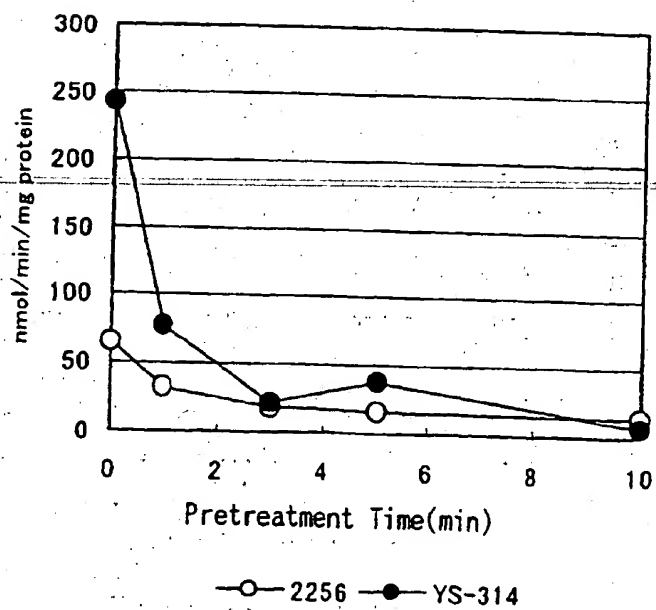


Fig. 9

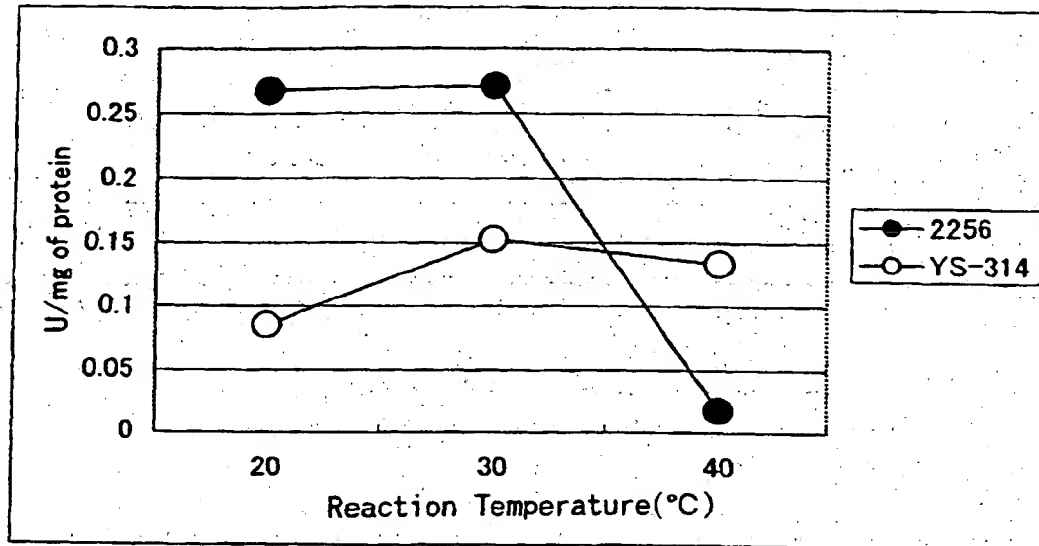


Fig. 10

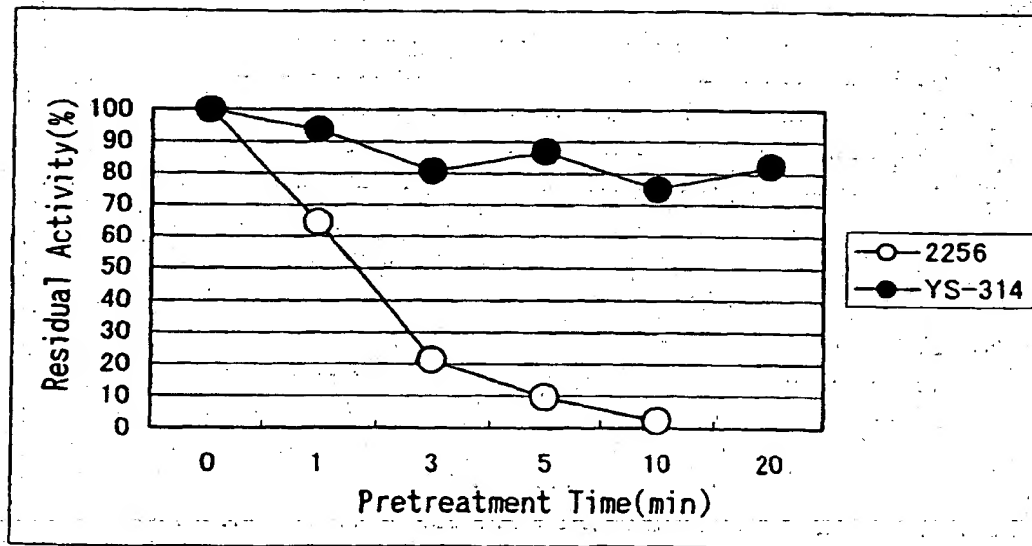


Fig. 11

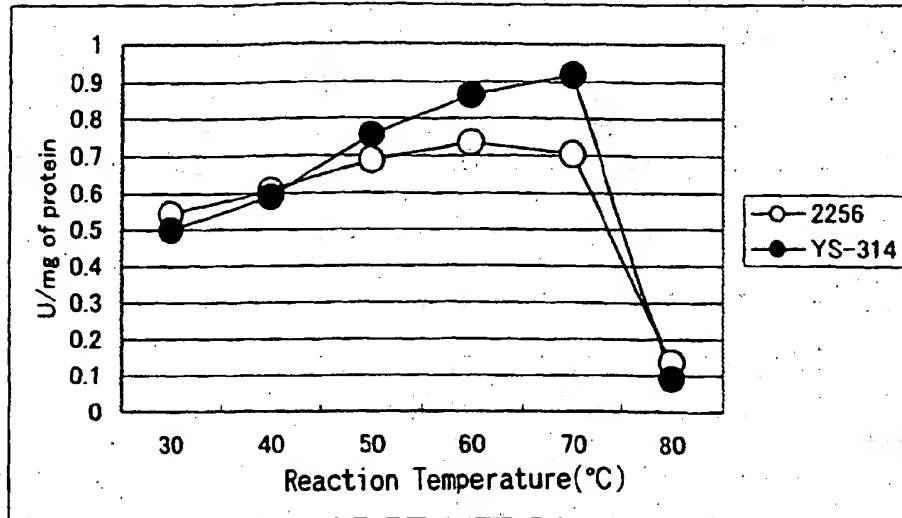


Fig. 12

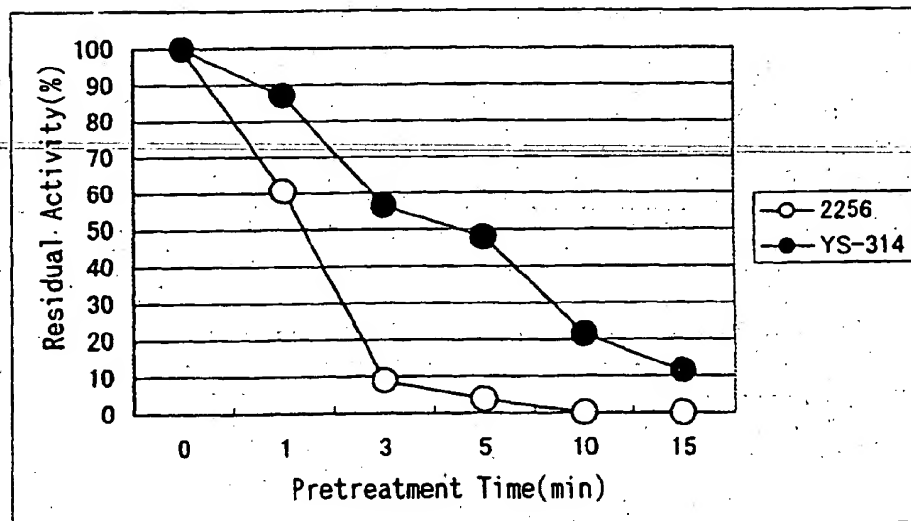


Fig. 13

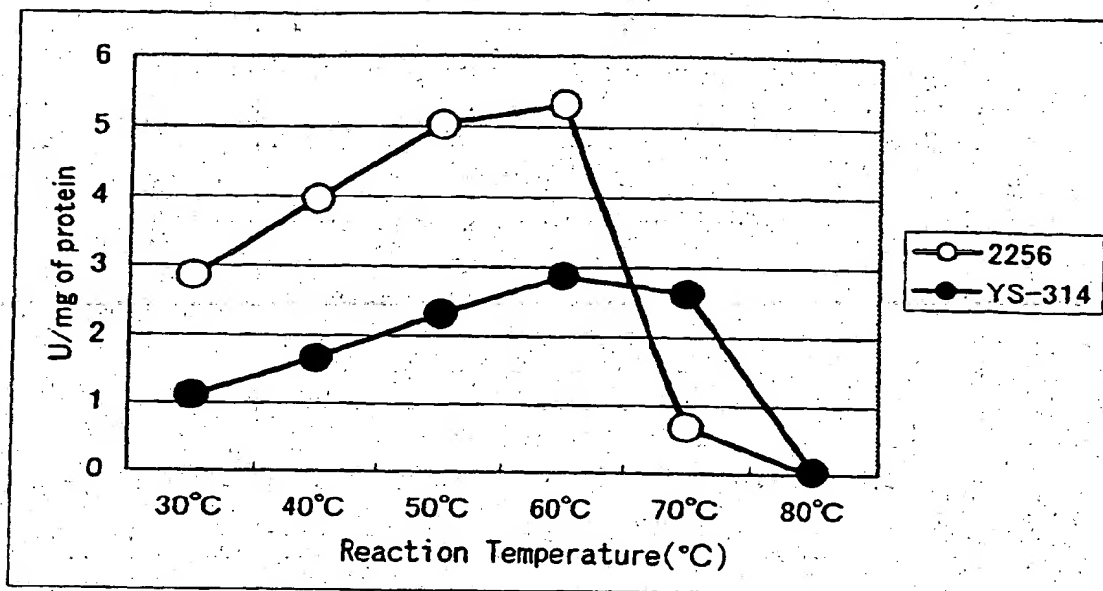
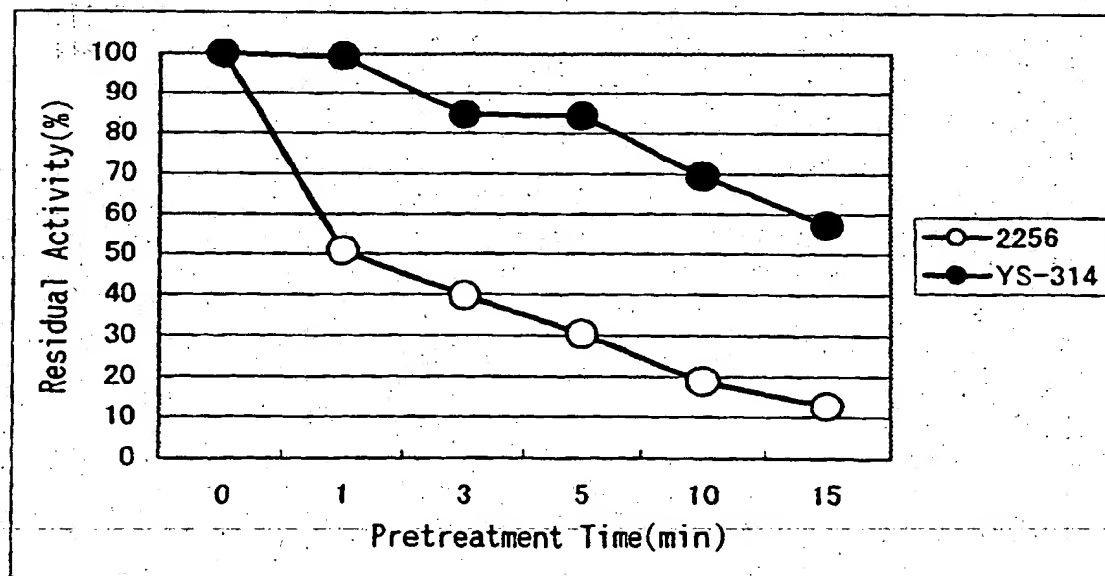
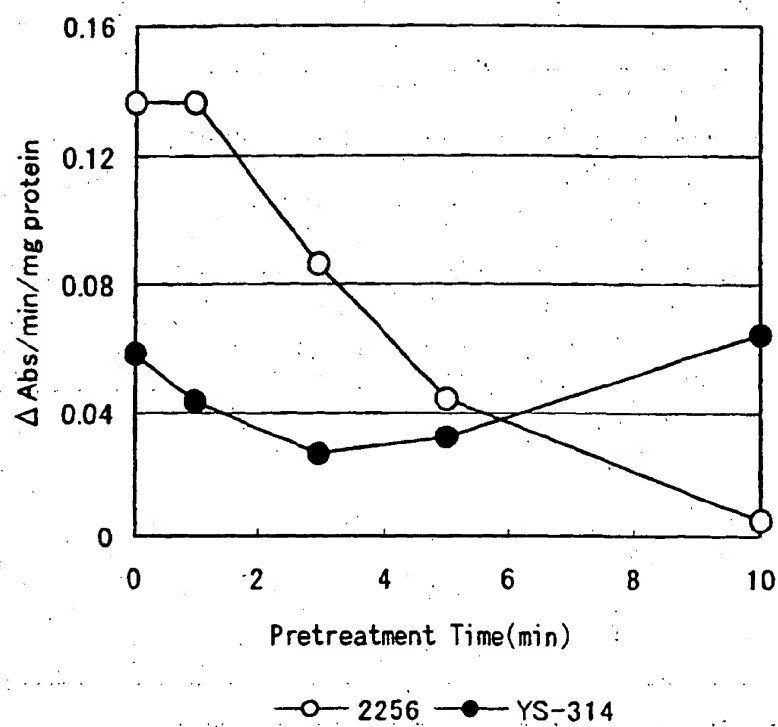


Fig. 14



*Fig. 15*

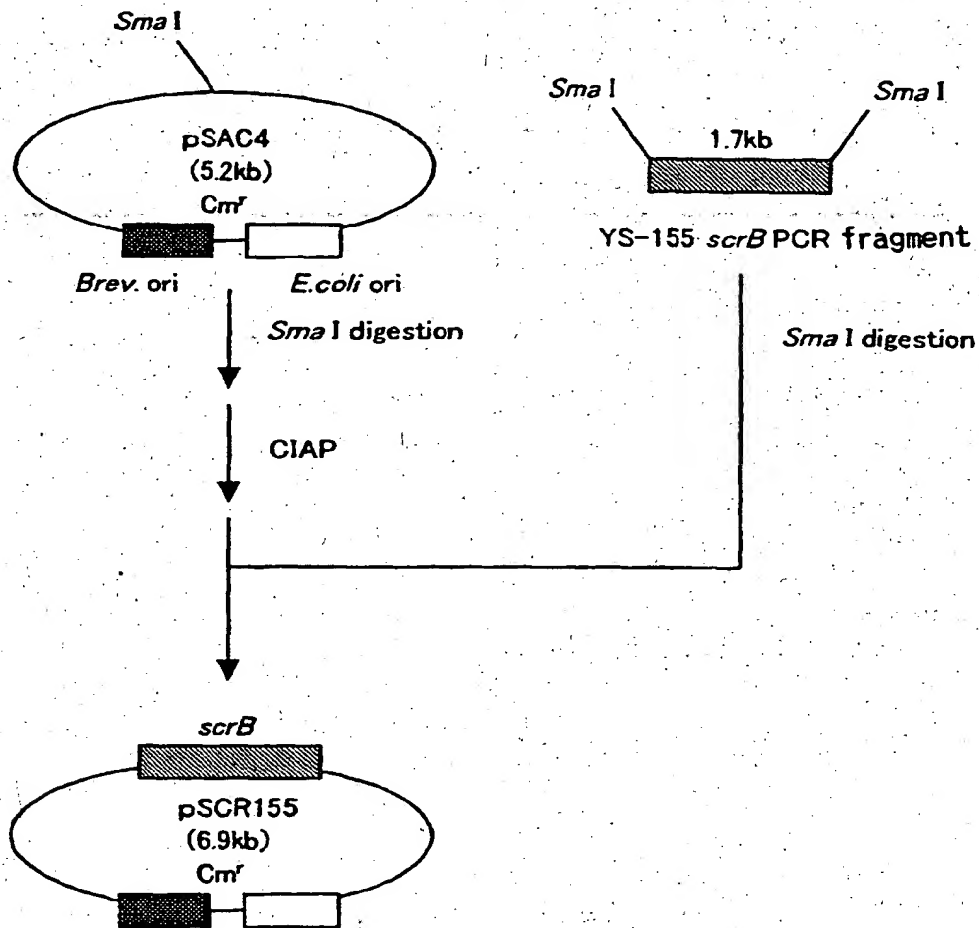


Fig. 16

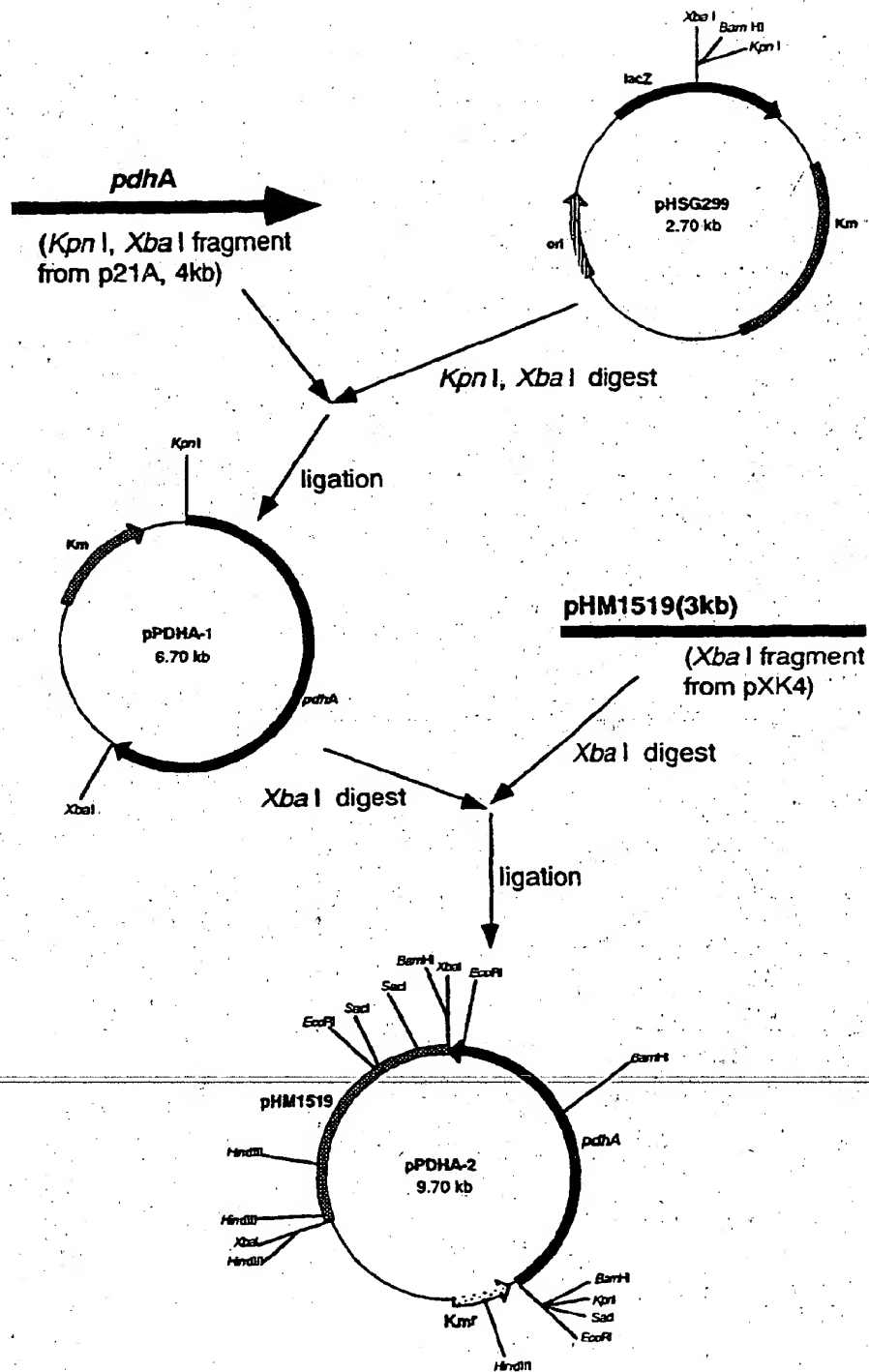


Fig. 17

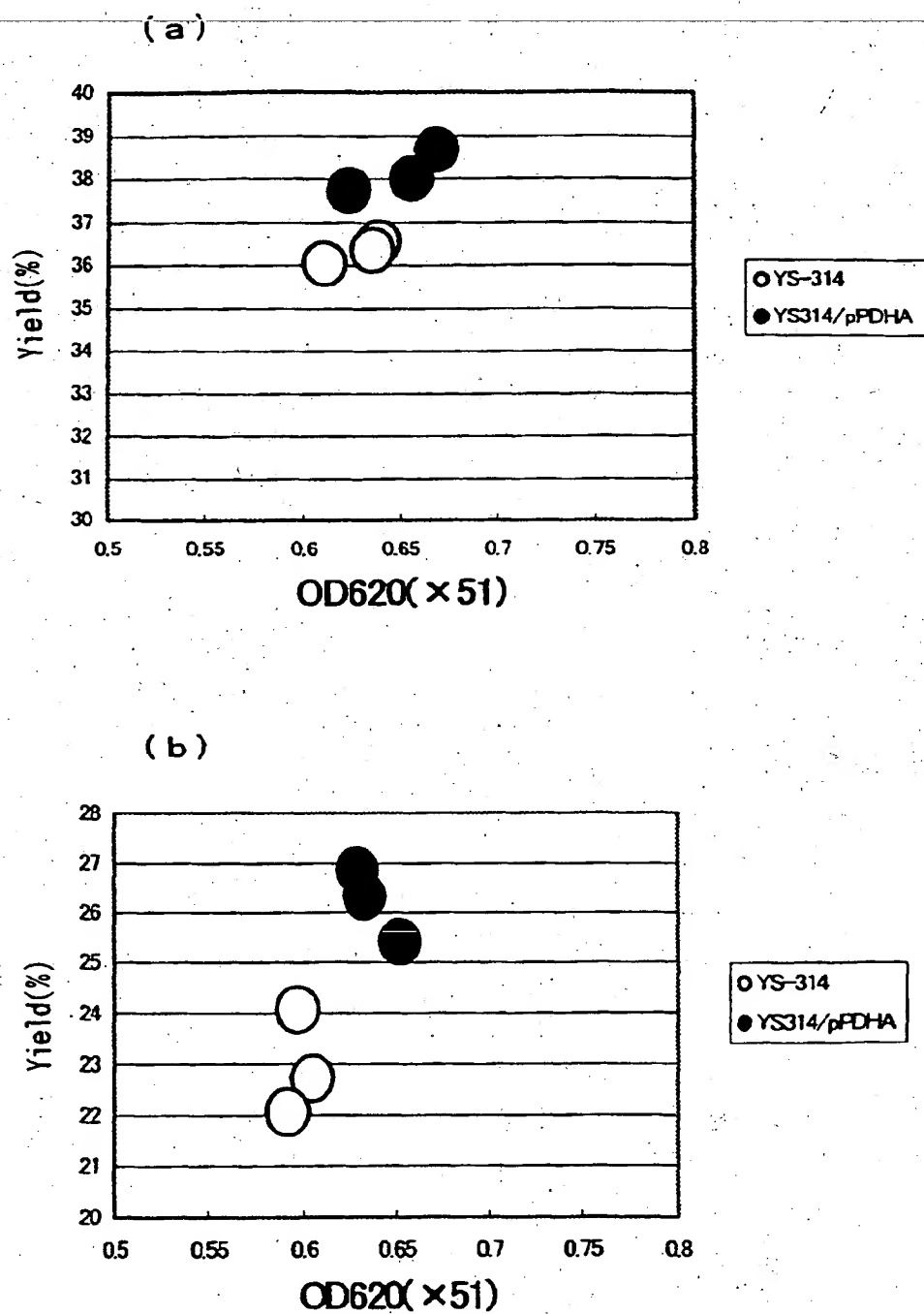


Fig. 18

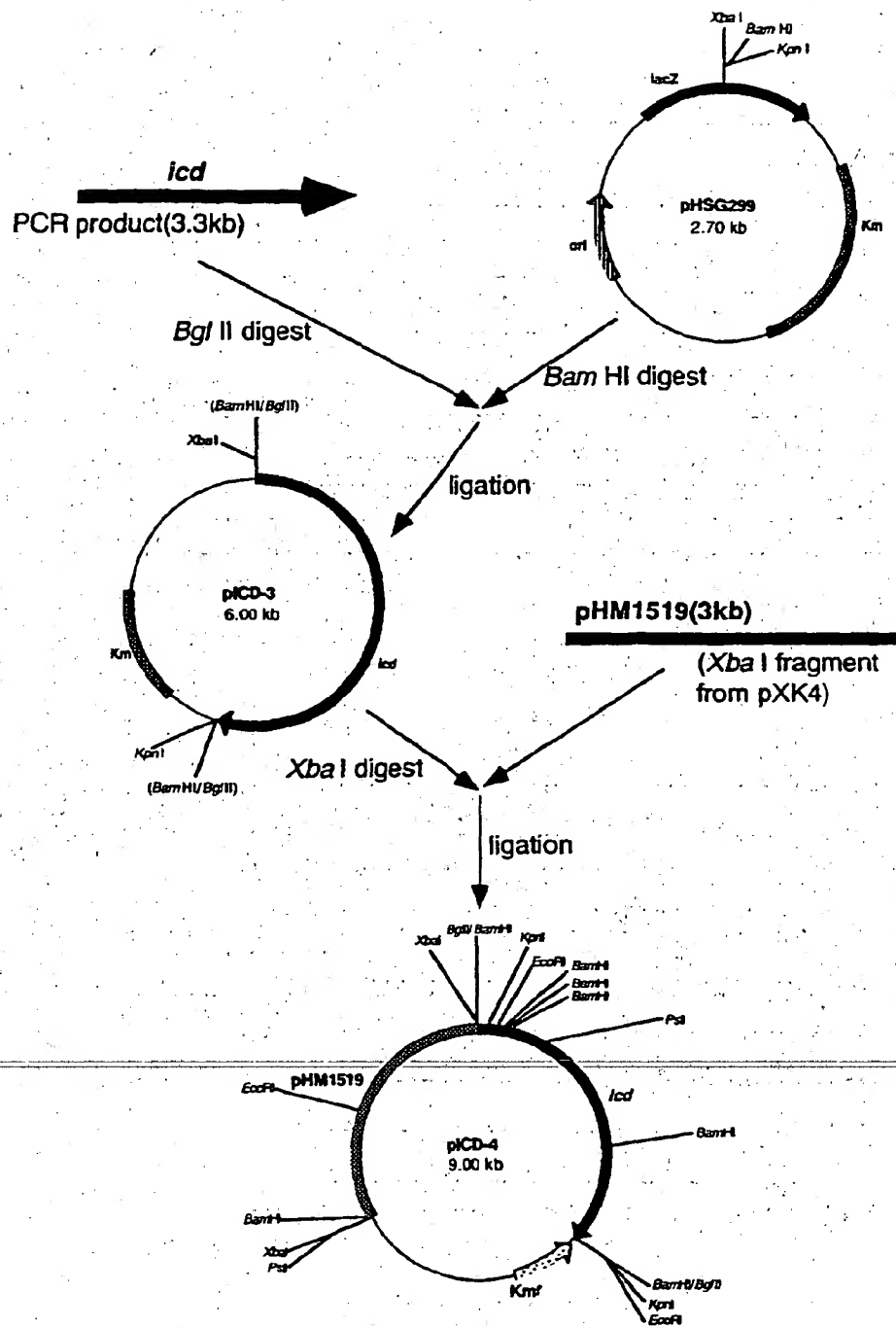


Fig. 19

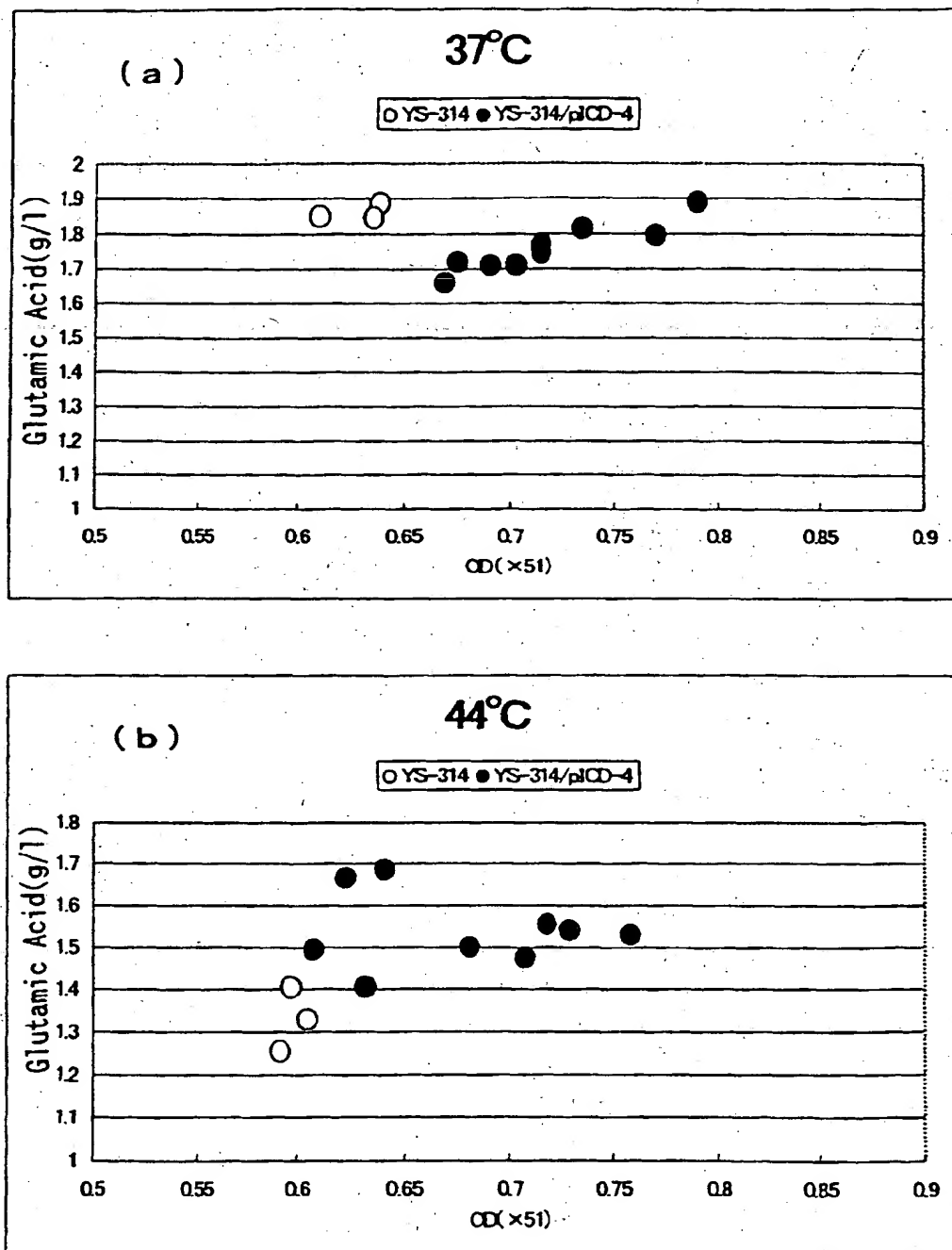


Fig. 20

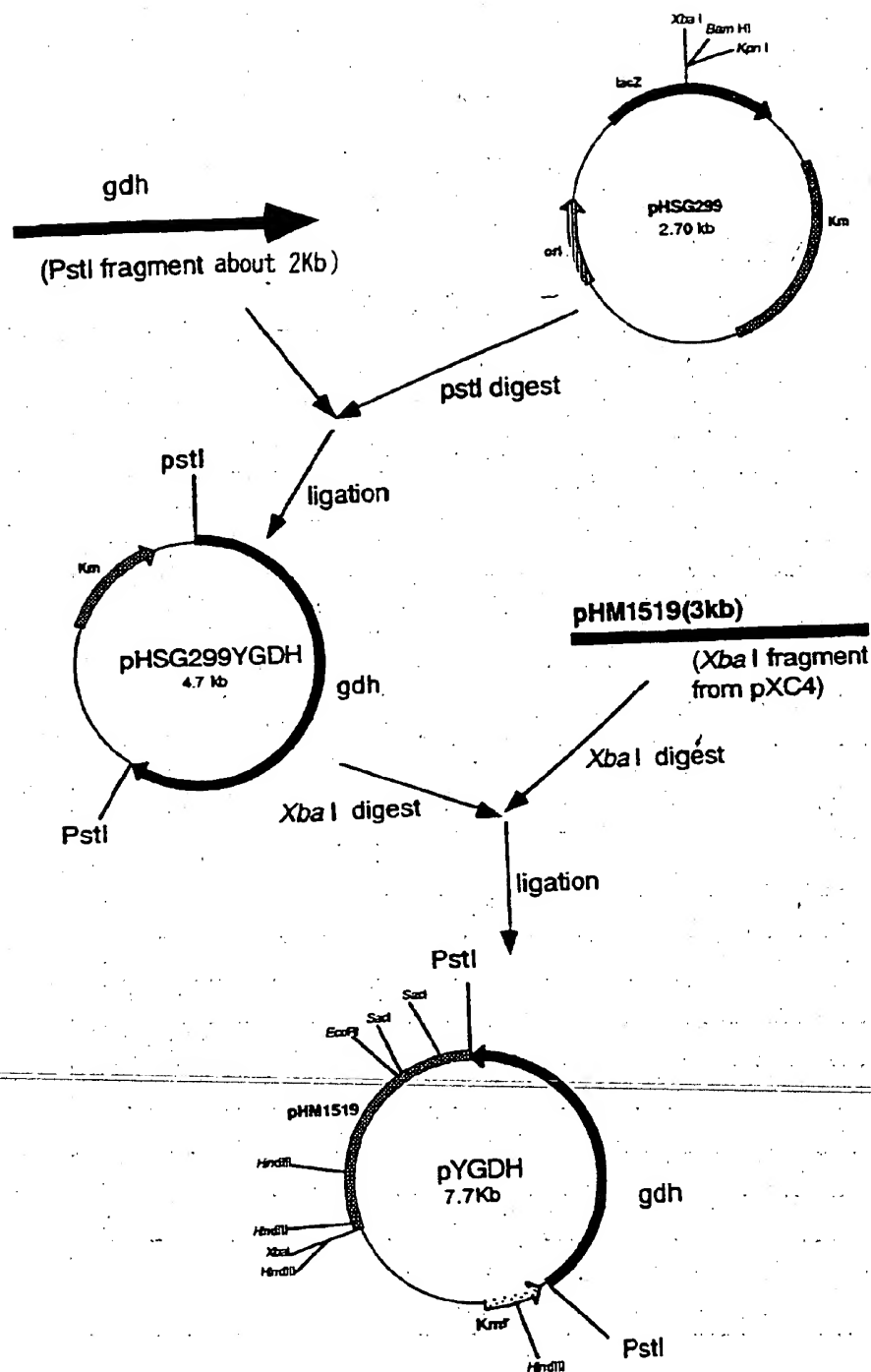


Fig. 21

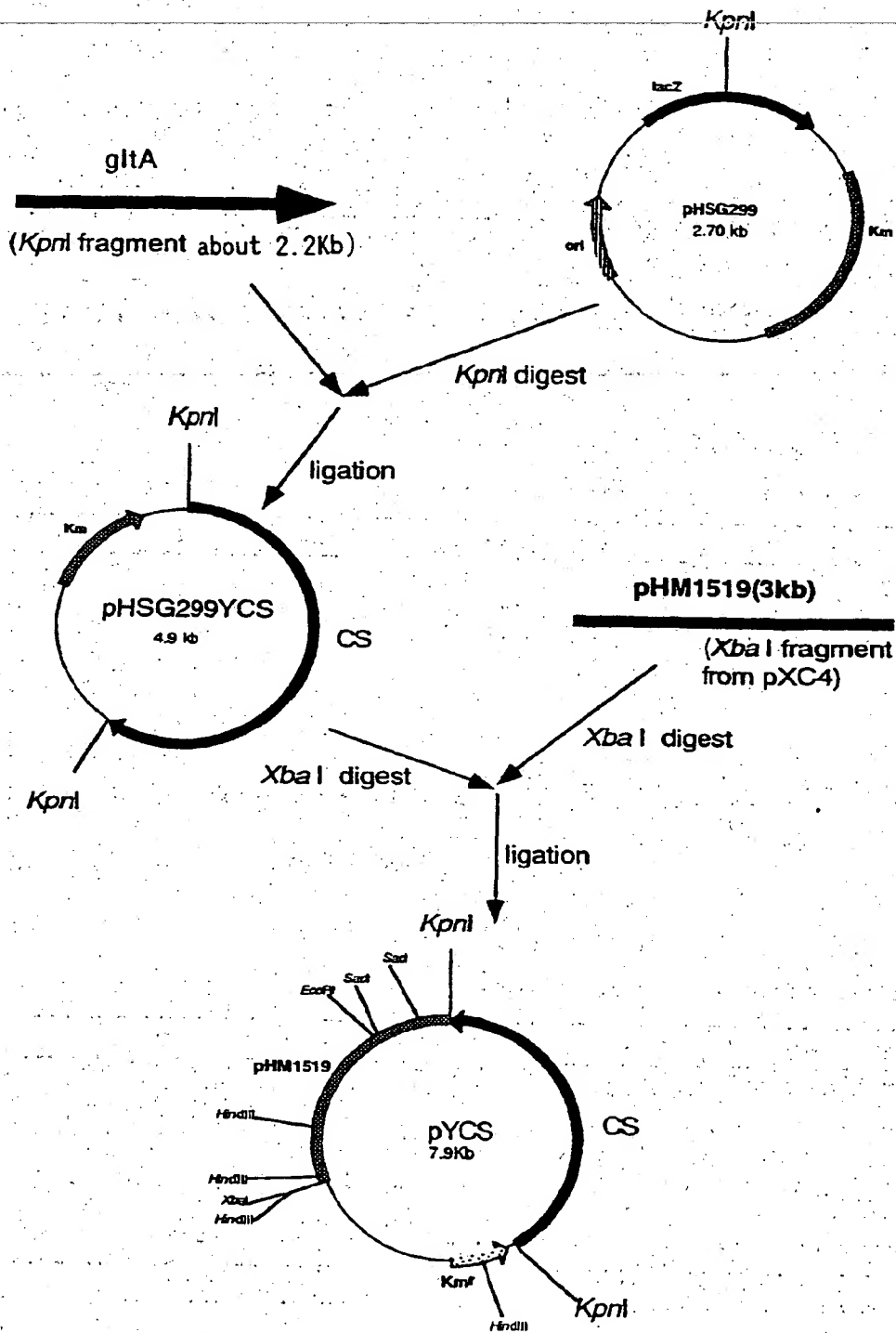


Fig. 22

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP00/06913

A. CLASSIFICATION OF SUBJECT MATTER Int. Cl. ⁷ C12N15/60, C12N15/54, C12N15/53, C12N15/31, C12N15/56, C12N9/88, C12N9/12, C12N9/04, C07K14/34, C12N9/26, C12P13/04 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) Int. Cl. ⁷ C12N15/60, C12N15/54, C12N15/53, C12N15/31, C12N15/56, C12N9/88, C12N9/12, C12N9/04, C07K14/34, C12N9/26, C12P13/04 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) JICST FILE (JOIS), WPI (DIALOG), BIOSIS (DIALOG), MEDLINE (STN), EMBL/DBJ/Genbank/PIR/Swissprot/Geneseq		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JP, 7-63383, B2, (Ajinomoto Co., Inc.), 12 July, 1995 (12.07.95), & FR, 2612937, A & US, 5250434, A & AU, 8811614, A & BR, 8801289, A & KR, 9606580, A	1-49
Y	JP, 4-4887, A (Ajinomoto Co., Inc.), 09 January, 1992 (09.01.92), & FR, 2661191, A & US, 5250423, A	1-49
Y	Microbiology, Vol.144 [5] (1998), K. Takai et al., "ppc, the gene for phosphoenolpyruvate carboxylase from an extremely thermophilic bacterium, <i>Rhodothermus obamensis</i> : Cloning, sequencing and overexpression in <i>Escherichia coli</i> ", pp.1423-1434	1-49
Y	JP, 5-56782, A (Ajinomoto Co., Inc.), 09 March, 1993 (09.03.93), & EP, 530765, A2 & US, 57700661, A & CA, 2077308, A & US, 5439822, A & TW, 260709, A & DE, 69217144, B	1,17,18,49
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 19 December, 2000 (19.12.00)		Date of mailing of the international search report 26 December, 2000 (26.12.00)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.		Authorized officer Telephonic No.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP00/06913

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Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO, 92/18635, A1 (Commonwealth Sci. & Ind. Res. Org.), 29 October, 1992 (29.10.92), & ZA, 9202761, A & AU, 9215771, A & NZ, 242370, A Fig. 5; Table 4	1, 17, 18, 49
Y	Gene, Vol. 145 [1] (1994) D. Weweecke et al. "Cloning and sequence analysis of the gene encoding isocitrate lyase from <i>Rhodococcus fascians</i> " pp. 109-114	1, 17, 18, 19
Y	Arch. Microbiol., Vol. 166 [2] (1996) W. Jager et al. "A <i>Corynebacterium glutamicum</i> gene encoding a two-domain protein similar to biotin carboxylases and biotin-carboxyl-carrier proteins" pp. 977-984	2, 19, 20, 49
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Y	WO, 96/32484, A2 (Arch. Dev. Corp.), & EP, 820514, A1 & AU, 9655432, A & US, 5910626, A Claim 32; sequence No. 8	2, 19, 20, 49
Y	Biosci. Biotechnol. Biochem., Vol. 60 (1996), E. Kimura et al., "Molecular cloning of a novel gene, <i>dtSR</i> , which rescues the detergent sensitivity of a mutant derived from <i>Brevibacterium lactofermentum</i> " pp. 1565-1570	3, 4, 21-24, 49
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Y	JP, 10-234371, A (Ajinomoto Co., Inc.), 08 September, 1998 (08.09.98) (Family: none)	3, 4, 21-24, 49
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Y	J. Bacteriol., Vol. 178 (1996) A. M. Alves et al., "Characterization and phylogeny of the <i>pfp</i> gene of <i>Amycolatopsis methanolica</i> encoding PPI-dependent phosphofructokinase" pp. 149-155	5, 25, 26, 49
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP00/06913

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Y	J. Bacteriol., Vol.177(1995) W. Kronmeyer et al. "Structure of the <i>gluABCD</i> cluster encoding the glutamate uptake system of <i>Corynebacterium glutamicum</i> ", pp.1152-1158	7, 29, 30, 49
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Y	JP, 2-291276, A (Degussa AG.), 18 April, 1990 (18.04.90), & EP, 358940, A1 & GB, 2223754, A & DE, 68924227, B	10, 35, 36, 49
Y	JP, 11-196887, A (Mitsubishi Chemical Corporation), 27 July, 1999 (27.07.99) (Family: none)	10, 35, 36, 49
Y	JP, 8-66189, A (Mitsubishi Chemical Corporation), 12 March, 1996 (12.03.96) (Family: none)	10, 35, 36, 49
Y	MIKROBIOLOGIA, Vol.56[5](1987), M. P. Ruklish et al., "The functioning of the tricarboxylic acid cycle in <i>Brevibacterium flavum</i> and <i>Micrococcus glutamicus</i> ", pp.759-763	11, 37, 38, 49
Y	J. Bacteriol., Vol.175(1993), J. M. Mengaud et al., "The major iron-containing protein of <i>Legionella pneumophila</i> is an aconitase homologous with the human iron-responsive element-binding protein" pp.5666-5676	11, 37, 38, 49
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Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J. Bacteriol., Vol.177(1995), B. J. Eikmanns et al., "Cloning, sequence analysis, expression, and inactivation of the <i>Corynebacterium glutamicum</i> <i>icd</i> gene encoding isocitrate dehydrogenase and biochemical characterization of the enzyme", pp.774-782	12,39,40,49
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Y	WO, 95/34672, A1 (Ajinomoto Co., Inc.), 21 December, 1995 (21.12.95), & US, 5977331, A & EP, 771879, A1 Claims; sequence Nos. 1, 2	14,43,44,49
Y	Mol. Microbiol., Vol.,6(1992), E. R. Boermann et al., "Molecular analysis of the <i>Corynebacterium glutamicum</i> <i>gdh</i> gene encoding glutamate dehydrogenase", pp.317-326	15,45,46,49
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Y	Microbiology, Vol.140(1994), B. J. Eikmanns et al., "Nucleotide sequence, expression and transcriptional analysis of the <i>Corynebacterium glutamicum</i> <i>gluA</i> gene encoding citrate synthase", pp.1817-1828	16,47,48,49
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Y	JP, 8-196280, A (Ajinomoto Co., Inc.), 06 August, 1996 (06.08.96), & EP, 724017, A2 & FR, 2729970, A & SK, 9600112, A & ZA, 9600656, A & BR, 9600268, A	6,27,28,49
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